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The pH and the amphoteric behavior of soils in relation to the Donnan equilibrium.

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From the Institute of Pedology.

Theoretical.

In our previous papers we have assumed that the saloids are incompletely dissociated, that the Ca-saloid is less dissociated than the Na-saloid and so forth. The charge, the power to imbibe water and other colloidal properties point that way. But it is also possible that the saloids, like most of the salts, are completely dissociated. It seems probable that monovalent groups on the surface of the acidoid are sitting so far apart that a complete association with a divalent cation is impossible. Thus the phosphate-bound Ca in a phosphoric acidoid (Fe- and Al-phosphates) is easily displaced by the alkali cations (MATTSON & KARLSSON 1938).

Yet, because of the very nature of the saloids, they may in *effect* be incompletely dissociated due to the fact that the micellar ions are within the range of attraction of the colloidal ion-complex. The interionic attraction must markedly reduce the activity coefficient (f) of the ions and this means a low activity (fc) in proportion to the concentration (c). The divalent ions, which may be assumed to be held nearer the surface than the monovalent ions, would therefore suffer the greatest reduction in their activity. The lower the activity of an ion in the micellar solution the greater will be the displacing power of that ions when added to the system.

Such a partial dissociation of all the ions would equally well serve to account for the variations in the apparent dissociation constants of the acidoid and basoid groups and for the position of the amphoteric points of soils in different salt solutions. An application of the mass law would lead to the same results as in the case of our previous assumption of a limited dissociation, provided that we substitute the activity in place of concentration.

But, even on the basis of a complete dissociation of the saloids, we can, in a general way, account for the phenomena here studied by an application of the mass law in the form of the Donnan equilibrium to the distribution of the free ions of the system, an application which has led to some very significant and interesting results.

The application of the Donnan equilibrium to the study of cation exchange in soils has been made by TERÄSVUORI (1930) and MÖLLER (1935). Valuable and interesting as this work is, it can hardly be considered to have passed the qualitative stage. DU RIETZ (1938) who used a ligno-sulphonic acidoid (a strong, completely dissociated acidoid) has been able to place the problem on a quantitative basis and thus greatly contribute to its solution. The results obtained by these workers leave no doubt that the classical mass law in its modern formulation can be applied even in the case of so complex a material as the soil. We shall, therefore, in the following apply the Donnan equilibrium to the amphoteric reactions of soils in order to show what consequences may be drawn and what conclusions may be arrived at.

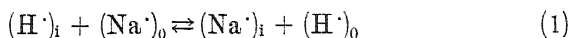
We are aware of the fact that an application of the Donnan equilibrium to amphoteric colloids is extremely complicated and we admit that it might seem difficult to understand how an amphoteric soil, which at the equi-ionic point binds large quantities of anions and cations, can possess an electrostatically attracted swarm of free ions of opposite sign of charge. Why do not these ions distribute themselves in pairs equally throughout the system, since the colloidal ions would compensate each

other like amphoteric »Zwitterionen»? But the acidoid and basoid ions may be too far apart to exert any appreciable interionic attraction. In this case these ions will attract the diffusible ions of opposite sign of charge and thus form an »amphoteric» ion atmosphere within which the anions and cations alternately dominate from point to point. The soil particle may also consist of a mosaic of acidoid and basoid clusters outside of which »clouds» of cations (over the acidoid areas) and of anions (over the basoid areas) would gather. This would especially be the case in the more heterogenous, less intimate mixtures of acidoids and basoids.

We shall, in what follows, assume that the amphoteric soil particle possesses, at and near the equi-ionic point, swarms of dissociated anions and cations.

The ions which form a swarm around the particles show no particular specifeness toward the colloidal ion as in the case of the formation of a crystal lattice or a slightly dissociated compound. Any other ion of the same sign of charge will compensate the colloidal ion equally well and will, therefore, when it comes near the surface allow the ion originally dissociated by the colloid to diffuse away. Within the micellar solution there is no difference between the ions »belonging» to a salt present in the system and the ions »belonging» to the colloidal ions. The micellar solution merely contains an excess of ions of opposite sign of charge to that of the colloidal ions. The concentration of the micellar solution (which contains the swarm ions together with the ions of the free electrolyte) is always greater than that of the outside solution but the two concentrations become almost equal at high concentrations. The composition of the micellar solution will depend upon the composition of the outside solution because there is a continual exchange of ions according to the mass law. For monovalent ions this exchange may be formulated as follows:

for an acidoid:



and for a basoid:

$$(\text{OH}')_i + (\text{Cl}')_0 \rightleftharpoons (\text{Cl}')_i + (\text{OH}')_0 \quad (2)$$

where the parenthesis stands for the ion activity and i and o signify that the ion is in the inside and the outside solution respectively.

The Donnan equilibrium then gives:
for the acidoid:

$$\frac{(\text{H}')_i \cdot (\text{Na}')_0}{(\text{H}')_0 \cdot (\text{Na}')_i} = K \quad (3)$$

and for the basoid:

$$\frac{(\text{OH}')_i \cdot (\text{Cl}')_0}{(\text{OH}')_0 \cdot (\text{Cl}')_i} = K \quad (4)$$

When the H and OH ions are displaced by divalent ions the corresponding equations become:

$$2(\text{H}')_i + (\text{Ca}''')_0 \rightleftharpoons (\text{Ca}''')_i + 2(\text{H}')_0 \quad (5)$$

$$2(\text{OH}')_i + (\text{SO}''_4)_0 \rightleftharpoons (\text{SO}''_4)_i + 2(\text{OH}')_0 \quad (6)$$

from which the corresponding Donnan expressions assume the following forms:

$$\frac{(\text{H}')_i^2 \cdot (\text{Ca}''')_0}{(\text{H}')_0^2 \cdot (\text{Ca}''')_i} = K \quad (7)$$

and

$$\frac{(\text{OH}')_i^2 \cdot (\text{SO}''_4)_0}{(\text{OH}')_0^2 \cdot (\text{SO}''_4)_i} = K \quad (8)$$

The Donnan distribution of the ions between the micellar and the outside solution may also be expressed as follows:
for the acidoid:

$$\frac{(\text{H}')_i}{(\text{H}')_0} = \frac{(\text{Na}')_i}{(\text{Na}')_0} = \frac{1}{\sqrt{(\text{Ca}''')_i}} \quad (9)$$

and for the basoid:

$$\frac{(\text{OH}')_i}{(\text{OH}')_0} = \frac{(\text{Cl}')_i}{(\text{Cl}')_0} = \frac{1 \sqrt{(\text{SO}''_4)_i}}{1 \sqrt{(\text{SO}''_4)_0}} \quad (10)$$

In studying the distribution of the ions of the free electrolyte between bentonite gel and the outside solution the following equations were used (MATTSON 1929 and 1932):

$$x^2 = y (y + z) \quad (11)$$

when all the ions are monovalent, e. g., Na-saturated bentonite in a NaCl solution:

$$\text{and} \quad x^3 = y^2 (y + z) \quad (12)$$

when the cation is divalent, e. g., Ca-saturated bentonite in a CaCl_2 solution.

Here x is the activity of the anions and cations of the free salt in the outside solution, y their activity in the micellar solution and z the activity in the micellar solution of the cation dissociated by the acidoid.

In terms of x , y and z equation (9) becomes¹

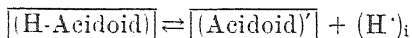
¹ Due to a confusion of terms MATTSON (1929) disregarded the valence when calculating the Donnan potential and erroneously concluded that the results in table 10 of the cited series were in agreement with the theory. In this experiment the ratios of x/y were about the same for the Cl and the SO_4 ions (which were both present) although x was in excess of y by a little over 0.011 in a total concentration of about one half normal. This is, as SCHOFIELD (1935) points out, «a serious discrepancy . . . in the one case where an internal check can be made».

The intention is here merely to correct an erroneous conclusion but it should also be pointed out that it is undoubtedly wrong to use so high concentrations in a study of the Donnan equilibrium. It is therefore not so surprising that the x/y ratios were the same for the two ions as that there should be any appreciable difference in the values of x and y . Or is the «negative absorption» due to two different causes: (1) to the fact that the «unfree» water does not act as solvent (in which case the valence would have no influence) and (2) to the Donnan distribution? Wiegner observed no negative adsorption when working on the basis of air dry material. We hope to repeat the experiment in table 10 in dilute solutions and once more try to gain some definite information concerning this very important problem.

$$\frac{y_{Na} + z_{Na}}{x_{Na}} = \frac{1}{1} \frac{y_{Ca} + z_{Ca}}{x_{Ca}} \quad (13)$$

In equations (9) and (10) the divalent ions enter as the square root of their activity. This means that a dilution, which affects the outside solution much more than the inside, will favor the ingo of the divalent and the outgo of the monovalent ions. That is, the relative displacing power of divalent ions will be greatly enhanced in dilute solutions whereas at high concentrations the displacing power of the two ions should be more nearly balanced provided the different saloids are completely or equally dissociated. It is the application of this law to the amphoteric behavior of soils which is the object of our present investigation.

It should perhaps be pointed out that the far greater displacing power of the H (and OH) ions in no way invalidates the application of this law. Whatever our views on the soil saloids might be it seems certain that the soil acidoids (and basoids) are dissociated to a limited extent. A low dissociation means a low activity in the micellar solution and the lower that this activity is the greater will be the displacing power of the H ions, because a correspondingly low activity in the outside solution will suffice to satisfy the relationship in equation (9). The free H ions are distributed according to the Donnan equilibrium the same as the other monovalent ions but their great displacing power is governed by the equilibrium:



The same applies to the OH ions of the soil basoids which are even weaker than the soil acidoids.

From the fact (1) that a soil simultaneously adsorbs large quantities of anions and cations at the equi-ionic point in the presence of a neutral salt, and from the fact (2) that in dilute solutions the divalent anions and cations displace the OH and H ions of the soil much more strongly than do the monovalent

anions and cations, whereas in stronger solutions the displacing power of the ions is more nearly balanced, we are lead to very significant and interesting conclusions.

If to an unsaturated soil, whose acidoid and basoid groups are present in equivalent proportion, we add increasing concentrations of a neutral salt solution containing a divalent cation and a monovalent anion, e. g. CaCl_2 , we ought to get the following results:

In low concentrations the Ca ions will displace considerably more H ions than the Cl ions will displace OH ions. In higher concentrations the two reactions will more nearly balance each other. The exchange acidity must therefore attain a maximum in dilute solutions and then decrease in higher concentrations.¹

If we add a solution containing a divalent anion and a monovalent cation, e. g. Na_2SO_4 , we ought to get a maximum exchange alkalinity in dilute solution.

To test this theory we selected a sample from the B horizon of an iron podzol (from Furudal, Dalarna), which had the same pH in water and in N/100 NaCl solution. We took this as an indication of an equivalence between the acidoid and basoid groups. The pH was determined by the glass electrode in water and in various concentrations of Na_2SO_4 up to N 2.0. The results, given in fig. 1, show that a concentration of N/50 gave a maximum exchange alkalinity equal to 0.52 pH unit

¹ A disturbing factor, especially when the acidoid content is high and the exchange acidity great, will be the dissolution of the basoid-bound Cl ions in the form of AlCl_3 which by hydrolysis gives HCl. Since this solution of Al (and Fe) is greater at a given pH the higher the concentration of salt it is clear that the exchange acidity, which thus comes from two sources, will be enhanced in the concentrated solution. To the extent that this dissolution takes place the exchange alkalinity resulting from a displacement of OH ions will be neutralized and this will partly or completely obscure the valence effect as above postulated according to the Donnan equilibrium. This applies to all the neutral salts but the effect will be smaller for salts of divalent anions and monovalent cations due to a higher pH and the coagulating effect of the divalent anions on the cationic sol complex.

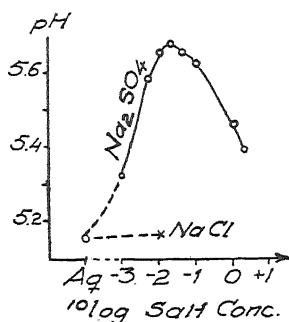


Fig. 1. The pH of the Furudal soil in water and in solutions of various concentrations of Na_2SO_4 .

whereas a 100 times stronger solution gave only a 0.24 unit higher pH than in water.

Determinations of the pH of various soils in water, in N/100 and in N/1 Na_2SO_4 gave the highest values in the N/1 solution when the basoid group dominated (lateritic subsoils), whereas the pH was highest in the N/100 solution when the equivalence between acidoids and basoids seemed to be balanced (lateritic surface soil and iron podzol samples from the

B-horizon). An excess of acidoids over basoids always resulted in an exchange acidity in both salt solutions (humus soils and gray soils). A little reflection will show that this is all in agreement with the theory. But the relationships are very complicated and will be more easily comprehended if we construct some theoretical curves analogous to the experimental curves.

Knowing neither the activities nor the concentrations of the ions in the micellar solution and knowing of no reliable method by which they may be determined in so complex systems as soils we shall not attempt a quantitative application of the above equations in order to find how the experimental results fit the theory. But we can make a qualitative application of the mass law to an amphoteric soil in at least two different ways. We can construct titration (dissociation) curves which are based on apparent acid and base dissociation constants assumed in accordance with the valence effect in the Donnan equilibrium and we can calculate this equilibrium on the basis of various assumed activities in the inside and outside solutions.

The more strongly an ion displaces the H ions of a soil acidoid the stronger will be the apparent dissociation constant of the acidoid and the greater will be the capacity of the soil

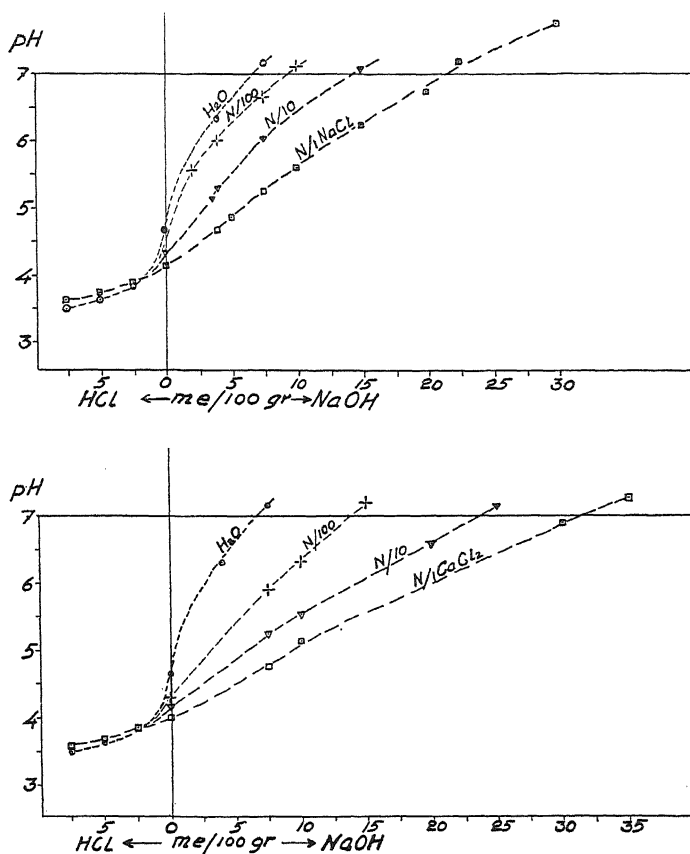


Fig. 2 and 3. The titration of the B₃ Haggbygget podzol in water and in N/100, N/10 and N/1 NaCl and CaCl₂.

to bind base at a given pH. The same applies to the basoids. Now it follows on the basis of the Donnan equation that, in low concentrations, divalent anions and cations must cause a much greater increase in the apparent basoid and acidoid dissociation constants than the monovalent ions whereas in high concentrations the increase will be more nearly the same.

That this is actually the case is shown in figures 2 and 3, which are taken from unpublished data by MATTSON and ERMAN.

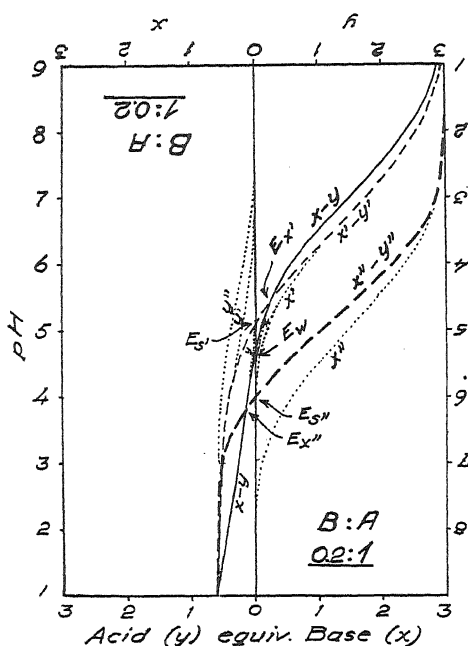


Fig. 4. The same as fig. 6 when basoid: acidoid = 0.2:1.

It will be seen that N/100 CaCl_2 causes a very much greater increase in the exchange acidity and in the capacity to bind base than does N/100 NaCl whereas the corresponding increments between N/10 and N/1 are greatest in the case of NaCl . We are presenting these curves merely to justify the assumptions we shall make below as a basis for the theoretical curves in fig. 6 and shall therefore not at this time discuss any other details in figures 2 and 3.

The question now arises how will this valence effect, in various combinations and concentrations of ions, affect the equi-ionic point and the point of exchange neutrality of the system?

To illustrate this we will first consider a system containing 1 mol of an acidoid H_3A and 1 mol of a basoid $\text{B}(\text{OH})_3$ whose apparent dissociation constants k_a and k_b and whose capacities

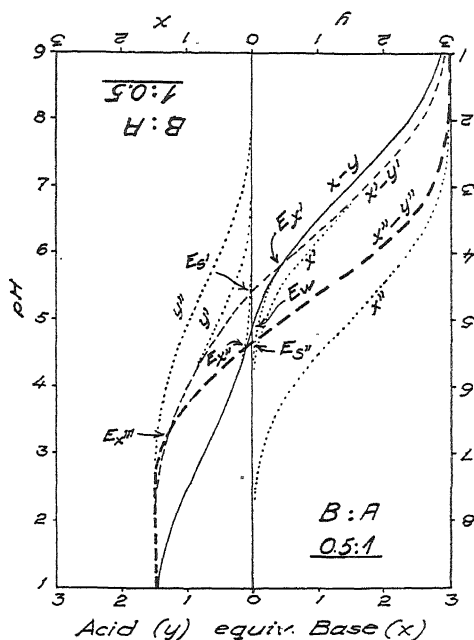


Fig. 5. The same as fig. 6 when basoid: acidoid = 0.5:1.

to bind base x^1 and acid y^1 respectively, when titrated in water, are as given under (A) and (B) in table 1. If instead of water we use a dilute solution of a salt of a divalent anion and a monovalent cation such as an alkali sulphate there will be a considerable increase in the k_b values whereas the k_a values will be much less affected [cf. (A') and (B') table 1]. In a strong solution of the same salt both the k_b and the k_a values will be greatly increased [cf. (A'') and (B'') table 1].

$$x = \frac{c}{1 + \frac{(H^+)}{k_a}}, \quad y = \frac{c}{1 + \frac{(OH^-)}{k_b}}$$

Table 1.

The theoretical »neutralization» of a system containing 1 mol of an acidoid H_3A and 1 mol of a basoid $B(OH)_3$.

Capacity to bind base (x) and acid (y) when:

(H^+) (OH^-)	10^{-0} 10^{-14}	10^{-1} 10^{-13}	10^{-2} 10^{-12}	10^{-3} 10^{-11}	10^{-4} 10^{-10}	10^{-5} 10^{-9}	10^{-6} 10^{-8}	10^{-7} 10^{-7}	10^{-8} 10^{-6}	10^{-9} 10^{-5}	10^{-10} 10^{-4}
(A) $k_{a_1} = 1 \times 10^{-6}$, $k_{a_2} = 1 \times 10^{-7}$, $k_{a_3} = 1 \times 10^{-8}$, $c = M/1$											
x					.01	.10	.60	1.50	2.40	2.90	2.99
(B) $k_{b_1} = 1 \times 10^{-10}$, $k_{b_2} = 1 \times 10^{-11}$, $k_{b_3} = 1 \times 10^{-12}$, $c = M/1$											
y	2.99	2.90	2.40	1.50	.60	.10	.01				
x-y	-2.99	-2.90	-2.40	-1.50	-.59	± .0	.59	1.50	2.40	2.90	2.99
(A') $k'_{a_1} = 1.78 \times 10^{-6}$, $k'_{a_2} = 1.78 \times 10^{-7}$, $k'_{a_3} = 1.78 \times 10^{-8}$, $c = M/1$											
x'					.02	.17	.81	1.74	2.58	2.94	2.99
(B') $k'_{b_1} = 3.16 \times 10^{-9}$, $k'_{b_2} = 3.16 \times 10^{-10}$, $k'_{b_3} = 3.16 \times 10^{-11}$, $c = M/1$											
y'	3.00	3.00	2.97	2.73	1.97	1.03	.27	.03			
x'-y'	-3.00	-3.00	-2.97	-2.73	-1.95	-.86	.54	1.71	2.58	2.94	2.99
(A'') $k''_{a_1} = 1 \times 10^{-4}$, $k''_{a_2} = 1 \times 10^{-5}$, $k''_{a_3} = 1 \times 10^{-6}$, $c = M/1$											
x''			.01	.10	.60	1.50	2.40	2.90	2.99	3.00	3.00
(B'') $k''_{b_1} = 3.16 \times 10^{-8}$, $k''_{b_2} = 3.16 \times 10^{-9}$, $k''_{b_3} = 3.16 \times 10^{-10}$, $c = M/1$											
y''	3.00	3.00	3.00	2.97	2.73	1.97	1.03	.27	.03		
x''-y''	-3.00	-3.00	-2.99	-2.87	-2.13	-.47	1.37	2.63	2.96	3.00	3.00
(x'-x)-(y'-y)	-.01	-.10	-.57	-1.23	-1.36	-.86	-.05	.21	.18	.04	
(x''-x)-(y''-y)	-.01	-.10	-.59	-1.37	-1.54	-.47	.78	1.13	.56	.10	.01
(x''-x')-(y''-y')			-.02	-.14	-.18	.39	.83	.92	.38	.06	.01

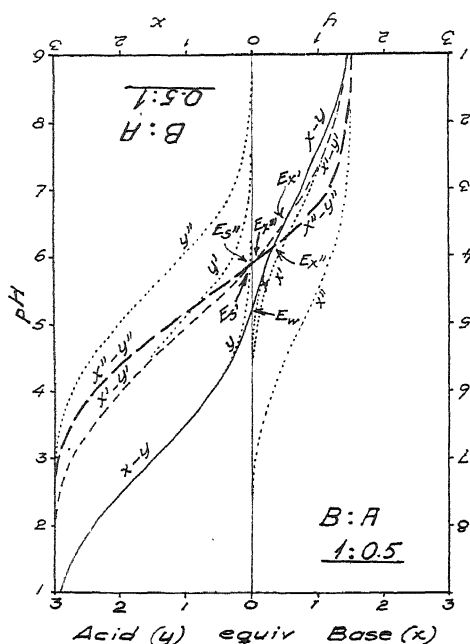


Fig. 7. The same as fig. 6 when basoid: acidoid = 1:0.5.

In terms of pK the assumed values for the apparent dissociation constants in water and in solutions of a neutral salt of the type M_2S'' are as follows:

Table 2.

	pK_{a_1}	pK_{a_2}	pK_{a_3}	pK_{b_1}	pK_{b_2}	pK_{b_3}
In water	6.0	7.0	8.0	10.0	11.0	12.0
In dilute solution	5.75	6.75	7.75	8.5	9.5	10.5
In concentrated solution . . .	4.0	5.0	6.0	7.5	8.5	9.5

By plotting the x and y values against the pH we get the curves in figures 6. The first notable thing is here the fact that the equi-ionic point ($x-y=0$) in the dilute solution E_s ,

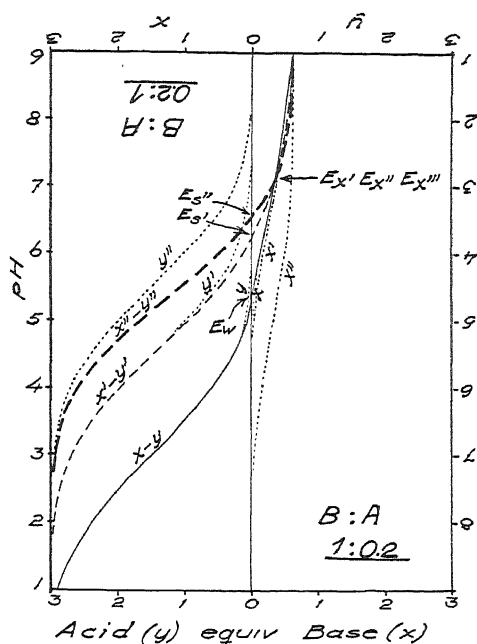


Fig. 8. The same as fig. 6 when basoid: acidoid = 1:0.2.

is not only above the equi-ionic point in water E_w but is also above the equi-ionic point in the concentrated solution $E_{s''}$. (Cf. the various values for x and y and $x-y$ in the column under $(H^+) = 10^{-5}$ in table 1.) A soil having the corresponding amphoteric properties would, in the completely unsaturated condition, yield a greater exchange alkalinity upon the addition of a smaller than a larger amount of the salt exactly as we have shown in fig. 1.

From this we can make the further deduction that if the soil originally contained an amount of salt corresponding (when suspended in water) to that of the dilute solution and we then added salt to correspond to the concentrated solution we would find that the salt caused a lowering of the pH. If, however, we decided to leach the soil before determining the pH in water and in the solution we would find that the salt caused

an elevation of the pH. In the first case we would find an exchange acidity and in the second an exchange alkalinity.

The differences in the *increments* produced by the salt in the capacity of the system to bind base x and acid y are given in the last three rows of table 1. Where these differences are positive the addition of the salt produces an exchange acidity, where negative an exchange alkalinity. The point of exchange neutrality is the pH where this difference equals zero and corresponds to the point of intersection of each pair of curves in fig. 6. In changing from water to the dilute solution and from water to the concentrated solution the points of exchange neutrality, E_x' and E_x'' respectively, are both on the alkaline side of the corresponding equi-ionic points in our assumed system. In changing from the dilute to the concentrated solution the point of exchange neutrality E_x''' occurs far down on the acid side.

Interpolated the various amphoteric points in table 1 and in fig. 6 are approximatively as follows:

	E_w	E_s'	E_s''	E_x'	E_x''	E_x'''
pH	5.0	5.62	5.27	6.10	5.37	4.40

This scattered position of the points of exchange neutrality will give rise to some peculiar and interesting phenomena. Suppose that we have a soil possessing the assumed amphoteric properties and suppose that soil to be saturated with base to the extent that its pH lies between the points E_x'' and E_x' . A small amount of the salt would then cause an elevation of the pH whereas a large amount would cause a lowering of the pH. In the first case an exchange alkalinity, in the second an exchange acidity (and somewhere in between an exchange neutrality).

We can now study the influence on the above system of a salt of a divalent cation and a monovalent anion, such as an alkaline earth chloride, merely by turning fig. 6 upside down

and changing the x values to y values and (in table 1) the plus signs to minus signs and vice versa. By writing the pH values as indicated in the inverted figure the k_a and k_b values will be the same, but since the cation is now the ions which has the greatest effect in dilute solution the apparent increase in the constants will be shifted from the one to the other. In terms of pk or values for the apparent dissociation constants in water and in solutions of a neutral salt of the type $M \cdot S'_2$ will now, on the basis of the same assumptions, be as follows:

Table 3.

	pk_{a_1}	pk_{a_2}	pk_{a_3}	pk_{b_1}	pk_{b_2}	pk_{b_3}
In water	6.0	7.0	8.0	10.0	11.0	12.0
In dilute solution	4.5	5.5	6.5	9.75	10.75	11.75
In concentrated solution . . .	3.5	4.5	5.5	8.0	9.0	10.0

The amphoteric points, interpolated on the inverted figure, have the following approximate values:

	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$
pH	5.0	4.38	4.73	3.90	4.63	5.60

The equi-ionic points in the salt solutions and the points of exchange neutrality have now moved to the opposite side of pH 5.0 ($= E_w$). The inverted figure shows therefore why the addition of a neutral salt of the type $M \cdot S'_2$ to an unsaturated soil, whose amphoteric properties corresponds to our theoretical system, must result in an exchange acidity, and why this acidity must be greater in a dilute than in a concentrated solution of the salt.

We shall now see how changes in the proportions of acidoids to basoids affect the position of the amphoteric points in the

different types of salt solutions when the various assumed constants remain the same as in the above discussed system.

To do this we need two sets of figures for each type of salt, one in which the ratio of acidoid to basoid is increased and one in which it is decreased. But since we can get the effect of one type of salt (e. g. $M \cdot S'_2$) by turning the figures, showing the effect of the other type of Salt (e. g. M_2S''), upside down we can get along with two sets and shall confine ourselves to two figures in each set which, together with fig. 6, gives us a series of five ratios.

Figures 4, 5, 6, 7 and 8 show the capacities to bind acid and base when the ratios of basoid : acidoid are 0.2 : 1, 0.5 : 1, 1 : 1 and 1 : 0.5 and 1 : 0.2 respectively, (a) in the upright position when the pk values are as shown in table 2, in water ($x-y$), in a dilute ($x'-y'$) and in a concentrated ($x''-y''$) solution of a neutral salt of the type M_2S'' , and (b) in the inverted position when the pk values are as shown in table 3, when the neutral salt is of the type $M \cdot S'_2$.

The relationships brought out in the figures will be more easily seen if we put all the amphoteric points together as in table 4.

The values in the E_w-E_s' column, table 4, are all negative under A and positive under B. This means that in the unsaturated systems the salt M_2S'' would, in dilute solution, always give rise to an exchange alkalinity whereas the salt $M \cdot S'_2$ would, in dilute solution, always yield an exchange acidity. This shows that, as the theory demands, *the valence effect dominates in dilute solutions.*

The values in the E_w-E_s'' column change from positive to negative (from exchange acidity to exchange alkalinity) as the proportion of the basoid group increases and the acidoid group decreases, and vice versa, in the case of both types of salt. *In concentrated solutions the ratio of acidoids to basoids must therefore be expected to dominate over the valence effect.*

In fig. 9 we have made an attempt to show the relationship graphically. It might seem absurd to plot curves on the basis

Table 4.

The various points in figures 4—8 (approximate, interpolated pH values).

A. Upright position of figures. pk values as in table 2. Neutral salt of the type M_2S'' .

Fig.	Basoid : Acidoid	E_w	E_s	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$	$E_w - E_{s'}^1$	$E_w - E_{s''}^1$	$E_{s'} - E_{s''}^1$
4	0.2 : 1	4.67	5.05	3.97	5.40	3.78	none	— .38	.70	1.08
5	0.5 : 1	4.93	5.42	4.64	5.82	4.58	3.25	— .49	.29	.78
6	1.1	5.00	5.62	5.27	6.10	5.37	4.40	— .62	— .27	.35
7	1 : 0.5	5.17	5.85	5.87	6.53	6.12	5.92	— .68	— .70	— .02
8	1 : 0.2	5.40	6.25	6.55	7.00	7.10	7.10	— .85	— 1.15	— .30

B. Inverted position of figures. pk values as in table 3. Neutral salt of the type $M'S'_2$.

Fig.	Basoid : Acidoid	E_w	E_s	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$	$E_w - E_{s'}$	$E_w - E_{s''}$	$E_{s'} - E_{s''}$
4	1 : 0.2	5.33	4.95	6.03	4.60	6.22	none	.38	— .70	— 1.08
5	1 : 0.5	5.07	4.58	5.36	4.18	5.42	6.75	.49	— .29	— .78
6	1 : 1	5.00	4.38	4.73	3.90	4.63	5.60	.62	.27	— .35
7	0.5 : 1	4.83	4.15	4.13	3.47	6.12	4.08	.68	.70	.02
8	0.2 : 1	4.60	3.75	3.45	3.00	2.90	2.90	.85	1.15	.30

of so indefinite quantities as »dilute» and »concentrated» solutions, but since we actually do obtain similar curves experimentally and since the figure does bring out the general theoretical relationship when the variables are as above assumed, we find the procedure justified inasmuch as the curves will give a much better picture than words and tables.

Fig. 9 shows that (1) when the group which is acted upon by the divalent ions is in great excess the deflection of the pH should

¹ Negative values = exchange alkalinity. Positive values = exchange acidity.

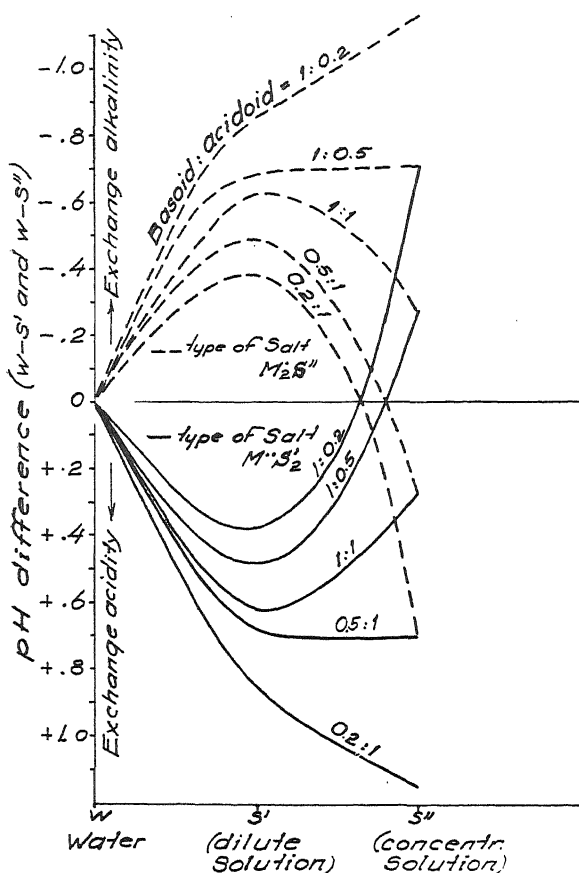


Fig. 9. The exchange alkalinity and exchange acidity of the various systems described in tables 1—4 and in figures 4—8 when acted upon by »dilute» and »concentrated» solutions of salts of the types M_2S' and $M'S'_2$.

be greater in a concentrated than in a dilute solution (cf. uppermost and lowest curves in fig. 9. Cf. also fig. 8); (2) when the proportion of the acidoid and basoid groups are more balanced the dilute solution should produce the greatest effect (cf. 1:1 ratio curves in fig. 9. Cf. also fig. 6); and (3) when the group which is acted upon by the monovalent ions is in great excess

the dilute and the concentrated solutions may cause an opposite deflection of the pH. (Cf. fig. 4 and 5.)

A study of fig. 4—8 will show that if a soil, which contains a higher proportion of acidoids than basoids, is saturated to a certain extent with bases, it will give the »regular» type of exchange reaction. No matter which salt we use we will get an exchange acidity and this acidity will increase with increasing concentration. The phenomena here discussed must therefore be studied on completely unsaturated soils, and preferably on soils having high equi-ionic points in order to avoid the disturbing effect of the dissolution of Al und Fe above alluded to.

The above application of the theory illustrates the relationship between the valence effect in the Donnan equilibrium and the ratio of acidoids to basoids in the colloidal complex. We have been discussing this relationship in terms of »dilute» and »concentrated» solutions but we have said nothing about the degree of dilution or concentration at which the valence effect will become most pronounced. We have said that a dilution favors the ingo of the divalent ions and the outgo of the monovalent ions. But the valence effect operates in the insides as well as in the outside solution. Why then does a dilution favor the ingo of the divalent ions? The question might seem unnecessary but we are asking it because the answer to it will pave the approach to the next problem.

When we dilute the system we dilute the outside solution much more than the inside solution because x and y in equation (13) (not to be confused with x and y in the tables and figures in this paper) are practically reduced in proportion to the dilution whereas z remains unchanged. It is because the outside solution becomes *relatively* diluted that the equilibrium is deflected toward a greater ingo of divalent ions. The factors which govern this relative dilution are (1) the concentration of acidoids and basoids and (2) the concentration (z) of the ions dissociated by the colloid. The concentration of the displaced ions in the outside solution as compared to the

Table 5.

The approximate theoretical displacement of H and OH ions at various concentrations of an unsaturated soil whose acidoid = basoid = (a) 100, (b) 10 and (c) 1 m.e. per liter, by neutral salts of the type $M'S'_2$ and M_2S'' when the activity (z) of the ions dissociated by the colloid is (I) N/1 and (II) N/10. (Cf. formula (9) and (10) and fig. 10).

Activity in the inside solution		Activity in the outside solution		
H' or OH'	M' or M' · S' or S''	H' or OH'	M' or S'	M' · or S''
n.	n.	n.	n.	n.
(I a) = 100 m.e./l., $z = N/1$.				
.9	.1	.01	.0011	1.23×10^{-5}
.8	.2	.02	.0050	1.25×10^{-4}
.6	.4	.04	.0266	.00178
.5	.5	.05	.0500	.005
.4	.6	.06	.0900	.0135
.2	.8	.08	.3200	.128
.1	.9	.09	.8100	.729
(I b) = 10 m.e./l., $z = N/1$.				
.9	.1	.001	.00011	1.23×10^{-7}
.8	.2	.002	.00050	1.25×10^{-6}
etc.
(I c) = 1 m.e./l., $z = N/1$.				
.9	.1	.0001	.000011	1.23×10^{-9}
.8	.2	.0002	.000050	1.25×10^{-8}
etc.
(II a) = 100 m.e./l., $z = N/10$.				
.09	.01	.01	.0011	1.23×10^{-4}
.08	.02	.02	.0050	1.25×10^{-3}
etc.
(II b) = 10 m.e./l., $z = N/10$.				
.09	.01	.001	.00011	1.23×10^{-6}
.08	.02	.002	.00050	1.25×10^{-5}
etc.
(II c) = 1 m.e./l., $z = N/10$.				
.09	.01	.0001	.000011	1.23×10^{-8}
.08	.02	.0002	.000050	1.25×10^{-7}
etc.

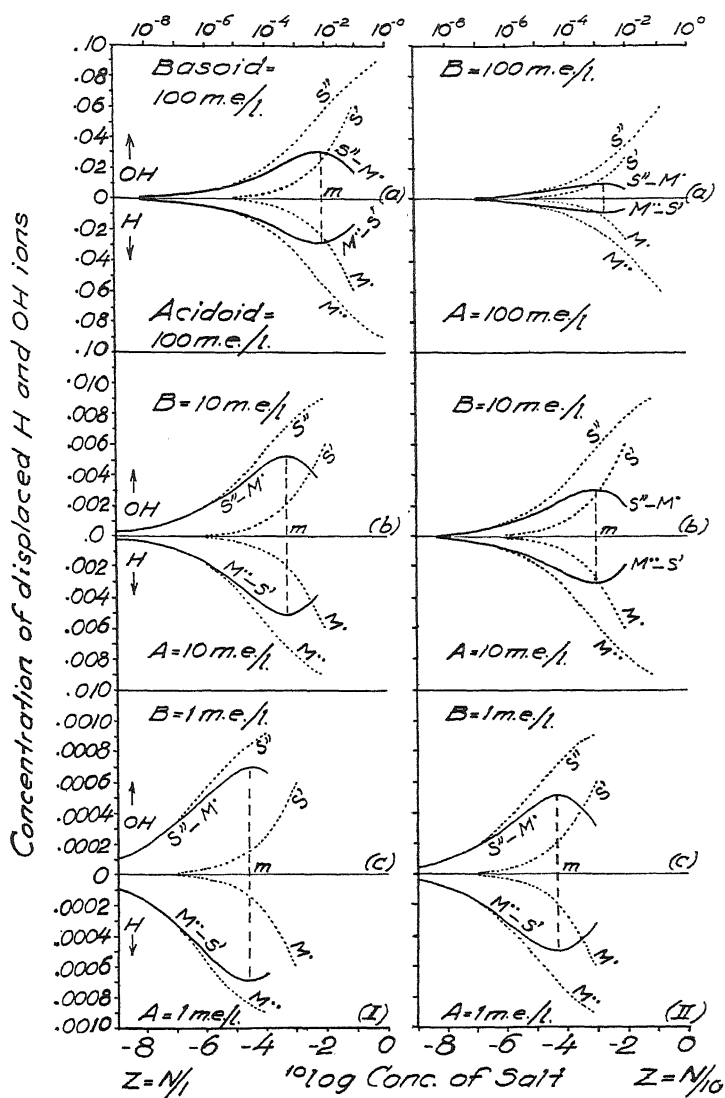


Fig. 10. The maxima in exchange alkalinity and exchange acidity in solutions of salts of the types M'_2S'' and $M''S'_2$ as related to the concentration of colloid (a, b and c) and to the concentration z of the ions dissociated by the colloid (I and II) as based on table 5.

concentration of the inside solution will be lower (1) the lower the concentration of the colloid (in terms of acidoids and basoids) and (2) the greater the concentration (z) of the dissociated ions. The more dilute the suspension of a soil and the higher the value of z the lower must be the concentration at which the valence effect will be most pronounced and the lower, therefore, the concentration at which maxima in exchange acidity and exchange alkalinity will occur.

The approximate relationship is brought out in table 5 and in fig. 10. The calculated concentrations (activities) are approximations in so far that we have put the inside concentration equal to a constant ($= z$) and ignored the concentration of the free electrolyte ($= y$ in equation 13). This does not involve any serious error as long as the values of x and y are small compared with z , but where the outside concentration (x) approaches that of the inside ($y+z$) as in II a table 5 and fig. 10 the results become misleading. With this exception the results are sufficiently accurate to bring out the true relationship.

The calculations apply to an unsaturated amphoteric soil whose acidoid/basoid ratio $= 1$ and which is assumed to be present in the concentrations of 100, 10 and 1 milliequivalents per liter, the values of z being in one case (I) put equal to $N/1$ and in the other (II) to $N/10$.

The assumption of a complete dissociation of the acidoid and basoid as well as of their saloids makes the concentration of the displaced H and OH ions very large. But this need not disturb us for we are here not concerned with the true concentrations of the displaced H and OH ions but with the relative proportions of the displaced ions.

Table 5 and fig. 10 show that the maxima m in exchange acidity and exchange alkalinity must occur at a lower concentration of the salt (1) the lower the concentration of the soil suspension (or the lower the colloid content of the soil) and (2) the higher the concentration z of the ions dissociated by the colloid (II a omitted).

The maxima in fig. 10 occur approximately at the following concentrations of the salts M_2S'' and $M'S'_2$:

Acidoid = basoid =	100	10	1 m.e. l
$z = N/1$.008	.00045	.000025
$z = N/10$	—	.0009	.00005

These positions of the maxima, especially those at the higher concentrations, are somewhat lower than the true theoretical positions, due to the use of z instead $y + z$.

The two maxima in each pair of curves in fig. 10 occur at the same concentration of salt because in each system we are assuming an equivalence between acidoids and basoids and assign the same value for z to both groups. In the soil we might have any proportion between these factors and the maxima in exchange acidity and exchange alkalinity may, therefore, occur at different concentrations of the salts. If, for example, the basoid content of a system be decreased, or the acidoid content increased, then the maximum in exchange acidity will not only become greater but must also be deflected to a higher salt concentration whereas the maximum in exchange alkalinity will become smaller and occur at a lower concentration of salt. If, on the other hand, the acidoid content of the system be decreased, or the basoid content increased, then the effect will be the opposite.

This is illustrated in fig. 11. The calculations apply to unsaturated soil suspensions whose acidoid/basoid ratios vary between 5.0 and 0.2 and whose acidoid and basoid concentrations are put at 2 to 10 milliequivalents per liter, the value of z being assumed to be equal to $N/1$ in every case.

If we compare the curves in fig. 11 with the curves in I b in fig. 10 we note that a decrease in basoids (= increase in acidoid/basoid ratio) causes the maximum in exchange alkalinity (m') to be smaller and to be deflected toward a lower

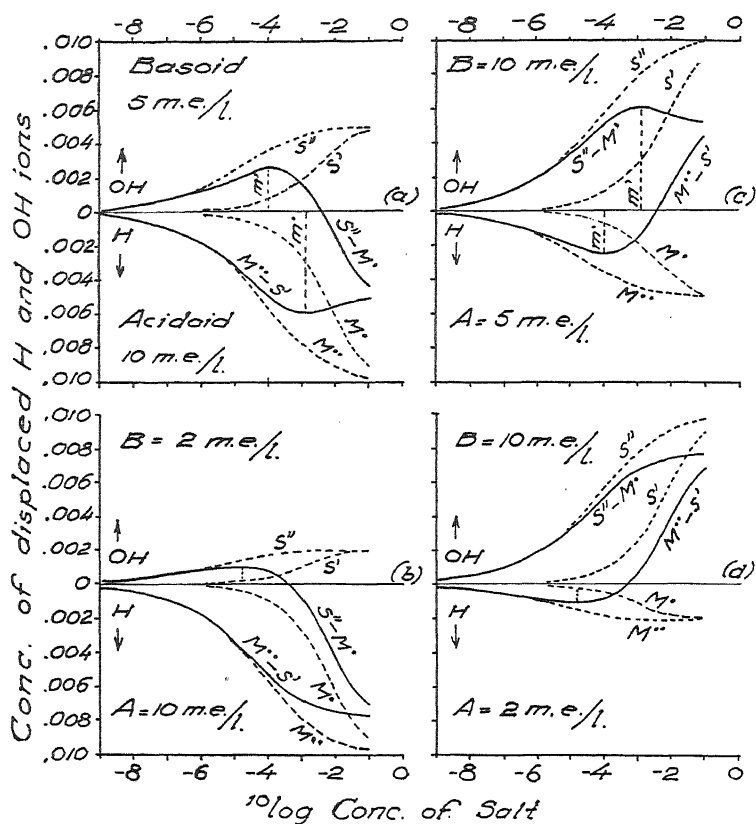


Fig. 11. The maxima in exchange alkalinity and exchange acidity as related to the acidoid/basoid ratio.

salt concentration whereas the maximum in exchange acidity (m') becomes larger and is deflected toward a higher concentration of salt. (Cf. curves in fig. 11 a and b.) A decrease in acidoids (= decrease in acidoid/basoid ratio) has the opposite effect. (Cf. curves in fig. 11 c and d.)

The maxima in exchange alkalinity in solution M_2S'' and in exchange acidity in solution $M'S'_2$ occur, in the different proportions of acidoid to basoid, approximately at the following equilibrium concentrations of the salts:

Fig.	11 b	11 a	10 I b	11 c	11 d
Acidoid m.e./l.	10	10	10	5	2
Basoid " "	2	5	10	10	10
M_2S'' n.	.000016	.00010	.00045	.00135	∞
$M \cdot S'_2$ "	∞	.00135	.00045	.00010	.000016

We shall now present our experimental data and show how we have applied the principles to a study of soil profiles.

Experimental.

For a systematic study of the application of the above principles to amphoteric soils we selected the following materials:

(1) Soil and three subsoils of a ferruginous laterite from Las Mesas plateau, Mayaguez, Puerto Rico. This is the same soil previously studied by MATTSON and GUSTAFSSON (1935) and which was kindly sent us by Dr. J. A. Bonnet, chief of the Division of Soils at the Insular Experiment station, Rio Piedras. The four samples, which we shall call Las Mesas, were taken at the following depths: I = 0—12", II = 13—23", III = 26—38" and IV = 43—58". Only samples I and IV have been titrated.

(2) Selected samples from a collection of 190 samples from a hydrologic podzol series of profiles taken in a 5 meter long trench which was dug from a wet depression into an adjoining hill of fine sandy material covering every transition from a peat podzol to the dry type of iron podzol. This series which is taken near the south end of lake Unden in the province of Västergötland is the object of an extensive investigation (MATTSON & LÖNNEMARK 1939). The profile series is expressed in the form of coordinate values of x and y, x = 0 cm. representing the wet end and x = 500 cm. the dry end of the

series whereas γ gives the depth of the sample below a horizontal line originating at the surface of the ground at the upper (dry) end of the series.

For our present work we selected three of the fifteen profiles included in the work of MATTSON and LÖNNEMARK viz. $x = 0$, $x = 280$ and $x = 500$ cm. From these three profiles we selected a horizontal series by taking the most strongly basoid sample in the B horizon of each profile. (Cf. fig. 24.) The titrations which required a considerable amount of material had to be made on a collection of large samples from a separate profile dug at a point $x = 400$ cm. The following samples were titrated: $B_2 = 27-35$ cm., $B_3 = 37-42$ cm., $B_4 = 50-55$ cm., $B_5 = 80-85$ cm. (Cf. fig. 15-19.)

Previous attempts to study the pH of laterites in different solutions by the quinhydrone and hydrogen electrodes have been unsuccessful but the possession of a glass electrode has apparently removed all difficulties. The laterites are particularly suitable for this kind of study, due to their high equi-ionic points which allow an extensive adsorption of anions before any appreciable dissolution of Al and Fe takes place. (Such dissolution causes the exchange acidity of a strong solution to appear greater than that of a dilute solution even where the reverse is true.)

Most laterites and all samples from the podzol B horizon are as good as completely unsaturated and need not be electro-dialysed. The Las Mesas subsoil contained practically no dialyseable bases but a not inappreciable quantity of SO_4 was present. This anion sits so tight that it becomes dialyseable only after the soil is made alkaline by ammonia. In the following experiment all samples were used in their natural condition.

Titration curves and the amphoteric points of soils.

Figures 12 and 13 give the titration curves of the Las Mesas I and IV in water, in N/1 and in N/100 Na_2SO_4 .

Without an application of the mass law and the valence effect as expressed by the Donnan equation these curves would, with respect to their intersections and relative position, be impossible to explain. But on the basis of the theory, as here developed, the explanation presents itself in the form of an almost perfect reproduction of the theoretical curves. Since the theoretical curves were constructed before the titrations were carried out we were surprised to find so good an agreement between theory and experiment.

A comparison of fig. 12 with fig. 6 and of fig. 13 with fig. 8 leaves no doubt as to the meaning of the relative position of the experimental curves: In Las Mesas I (surface soil) the activities of the acidoid and basoid groups are more nearly balanced, due to the presence of humus acidoids and to the less active (more dehydrated and aged) sesquioxides, whereas in Las Mesas IV the activity of the basoid groups is far in excess. The result is that, in Las Mesas I, the exchange alkalinity in the N/100 Na_2SO_4 solution is greater than in the N/1 solution, whereas in Las Mesas IV the exchange alkalinity, which is very great, increases with increasing concentration.

Because of its strong basoid character the Las Mesas IV ought to show the valence effect by yielding a greater exchange acidity in a dilute than in a concentrated solution of a salt of the type $\text{M} \cdot \text{S}'_2$. A preliminary experiment with BaCl_2 (cf. fig. 20, 0 % humus) gave a maximum in exchange acidity in a N/10 solution. The Las Mesas IV was therefore titrated in water and in N/10 and N/1 BaCl_2 with the results shown in fig. 14.

It will be noted that fig. 14 is an approximate counterpart to fig. 6 in the inverted position. This points to an approximate equivalence between the acidoids and basoids whereas the reaction with the sulphate points to a great excess of basoids. On the basis of the behavior of the soil in the sulphate solution we should expect fig. 14 to resemble fig. 4 in the inverted position, that is, the Las Mesas IV ought to yield

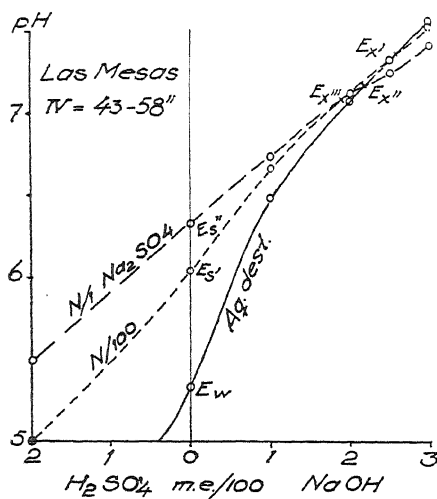
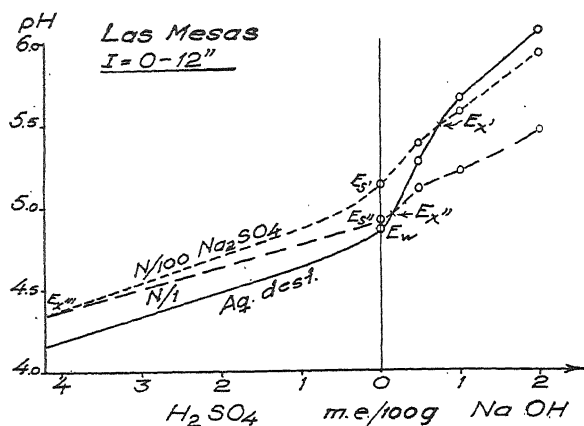


Fig. 12 and 13. The titration of the Las Mesas laterite.

an exchange acidity in the dilute and an exchange alkalinity in the concentrated $BaCl_2$ solution.

This anomaly in behavior we ascribe to the SO_4 ions which is present in the exchangeable form to the extent of 4.6 m.e. per 100 grams. These ions are, of course, precipitated by the

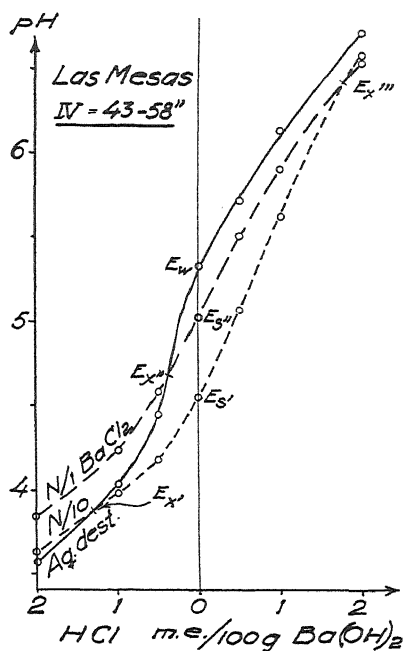


Fig. 14. The titration of the Las Mesas laterite.

Ba ions leading to a substitution of SO_4 for Cl ions. The monovalent Cl ions have a lower displacing power than the SO_4 ions. The place of the latter in the basoid complex will therefore be taken by OH as well as by Cl ions, and this leads to an exchange acidity, produced by an extramicrocellular reaction.

The presence of SO_4 in the laterite demands the use of CaCl_2 instead of BaCl_2 if this extra-microcellular reaction is to be avoided. But we had obtained better maxima in exchange acidity with Ba than with Ca ions and since the laterite yielded a pronounced maximum in BaCl_2 (N/10) we decided to use this salt even for this soil. We find the anomaly instructive and we shall later (cf. fig. 24) show evidence in support of our interpretation.

The amphoteric points of the three laterite samples in the solutions employed are as follows:

Fig.	Sample	Salt	E_w	E_s'	E_s''	E_x'	E_x''	E_x'''
12	Las Mesas I	Na_2SO_4	4.86	5.12	4.90	5.47	4.94	4.35
13	Las Mesas IV	"	5.33	6.04	6.33	7.37	7.16	7.08
14	Las Mesas IV	BaCl_2	5.33	4.55	5.03	3.87	4.68	6.43

It is interesting to note that the points of exchange neutrality of Las Mesas IV in Na_2SO_4 all occur above pH 7.

Figures 15 to 18 show the titration in water and in N/100 and N/1 Na_2SO_4 of the B_2 , B_3 , B_4 and B_5 samples of the Unden podzol ($x = 400$).

It will be noted that the activity of the basoid group decreases and that of the acidoid group increases (at least relatively) as we go from the B_2 to the B_5 horizon. Thus while the B_2 sample yields an exchange alkalinity (in the unsaturated condition) in the N/1 as well as in the N/100 solution, the B_3 sample yields an exchange alkalinity only in the N/100 solution and the B_4 and B_5 samples yield exchange acidity in both concentrations of the salt.

A comparison with the theoretical curves will show that fig. 15 resembles fig. 6 and that fig. 16 resembles fig. 5 with respect to the relative position of the amphoteric points. The titration curves of B_4 and B_5 have no theoretical counterpart among fig. 4 to 8. From the large amount of exchange alkalinity developed by these samples on the acid side of the points of exchange neutrality we know that they possess a considerable quantity of basoids. Why then does not the valence effect show itself here as in the case of the other samples by yielding an exchange alkalinity in the »dilute» solution?

The answer to this question is, as will be shown later, that the »dilute» solution was not dilute enough. The maximum

effect of the valence occurs here, where the basoid groups are relatively weak, at a lower concentration than $N/100 \text{ Na}_2\text{SO}_4$ (cf. fig. 19).

The amphoteric points of the four podzol samples in the solutions employed are as follows:

Fig.	Sample	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$
15	B ₂	5.01	5.41	5.15	7.71	5.46	4.93
16	B ₃	4.91	5.13	4.79	5.50	4.75	4.68
17	B ₄	4.70	4.65	4.45	4.55	4.39	4.38
18	B ₅	4.70	4.50	4.25	4.15	4.15	4.15

A great deal of work has been done to determine the various factors which influence the pH of the soil such as dilution, concentration of salt etc. In view of the above relationships we can readily appreciate why so little progress has been made. It is obvious that the soil reaction is so intimately related to the amphoteric nature of the soil complex and to the Donnan equilibrium that no student who does not embrace these ideas can hope to solve its problems. The fact that some of the $E_{s'}$ values are higher and some lower than the E_w values and that the $E_{x'}$ values vary between 4.15 and 7.71 in samples from the same soil profile could never be accounted for without the theory as here presented. The attempt by MATTSON and GUSTAFSSON to relate the point of exchange neutrality to the point of zero, net base saturation (base forming cations minus acid anions) will apparently fail since these points are variables related to other, indeterminable variables.

The maxima in exchange acidity and alkalinity.

Fig. 19 gives the pH in water and in various concentrations of BaCl_2 and Na_2SO_4 of the four Udden podzol samples shown in fig. 15—18.

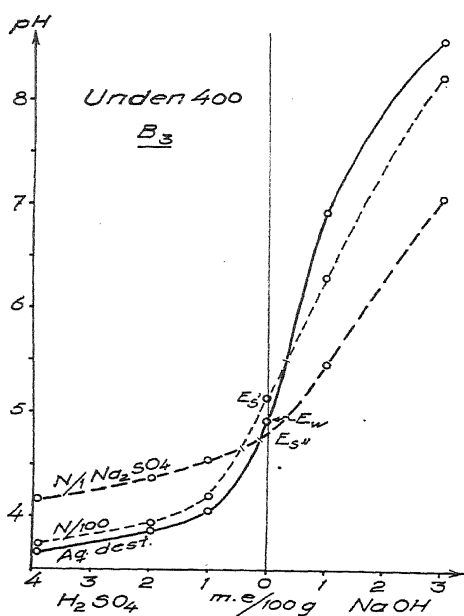
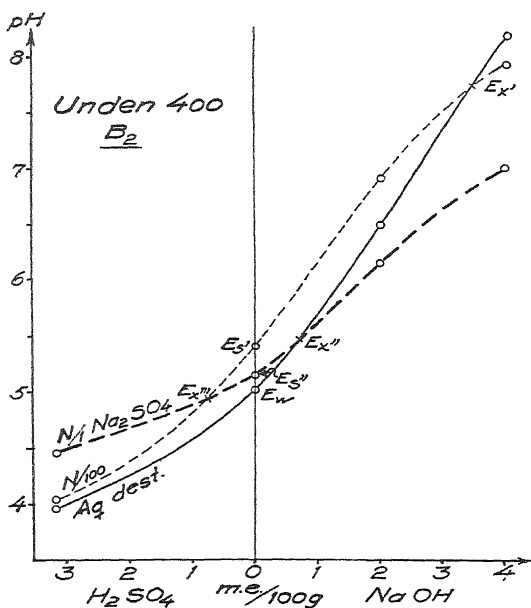


Fig. 15 and 16. The titration of the B samples of the Unden podzol profile 400.

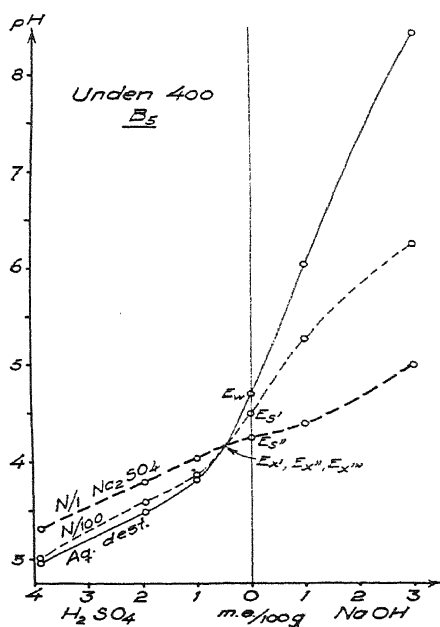
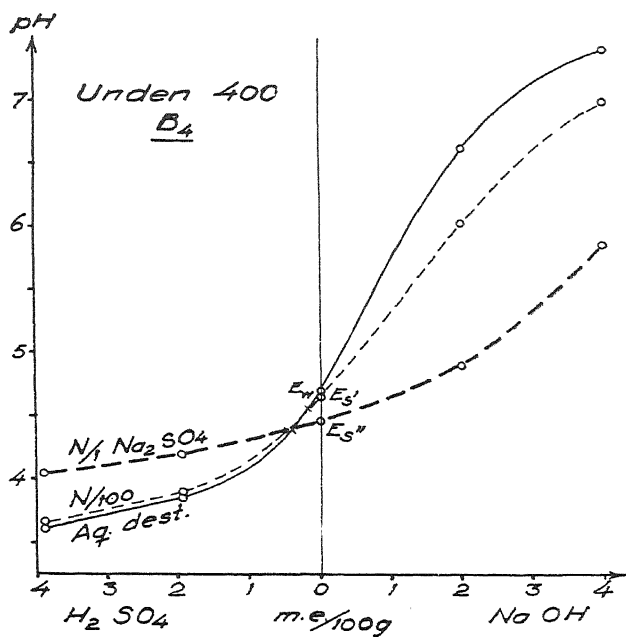


Fig. 17 and 18. The titration of the B samples of the Unden podzol profile 400.

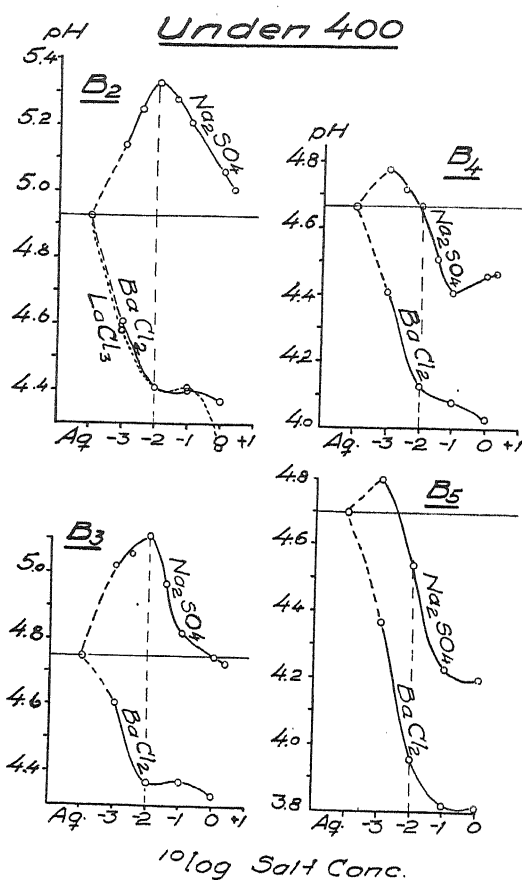


Fig. 19. The maxima in exchange alkalinity and exchange acidity in the B horizons of the Unden profile 400.

The following facts are of interest in connection with the theory:

1. The exchange alkalinity in Na_2SO_4 , which is greatest in B_2 , decreases with an increase in exchange acidity in BaCl_2 , which is greatest in B_5 .

2. As the exchange alkalinity is decreased its maximum is deflected toward a lower concentration of salt (from an initial

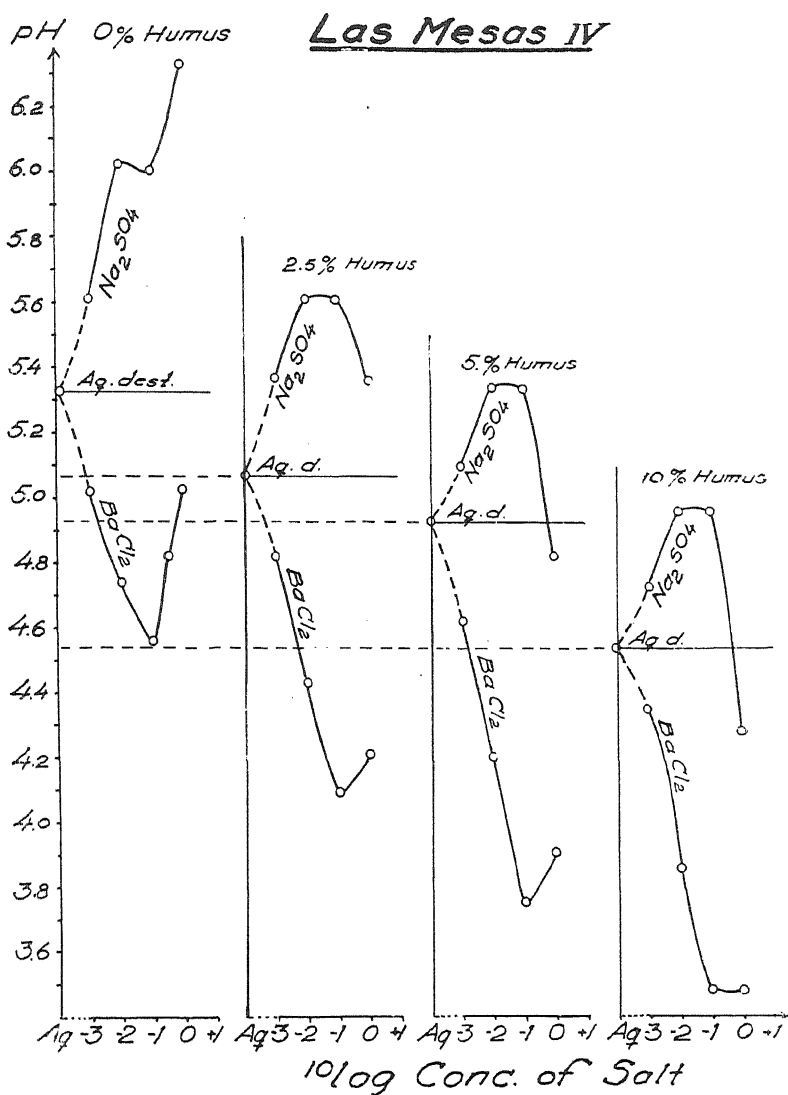


Fig. 20. The influence of humus on the exchange reactions of the Las Mesas laterite subsoil.

concentration of about $N/100$ in B_3 to $N/1000$, or less, in B_5). At the same time the maximum in exchange acidity is deflected toward a higher concentration of salt.

This is in agreement with the theory as illustrated in fig. 10 I b and fig. 11 a and b. It is obvious that when the acidoids greatly exceed the basoids the exchange alkalinity must reach a vanishing point at very low concentration of salt, and that the exchange acidity must assume a maximum in the most concentrated solution, exactly as shown in the figures.

We can now understand why we found no theoretical counterpart to fig. 17 and 18. Had we titrated the B_1 and B_5 samples in $N/1000$ instead of $N/100$ Na_2SO_4 we would have obtained a relative position of the curves similar to fig. 4. The »dilute» solution in fig. 17 and 18 was not dilute enough to show a maximum of the valence effect.

The decrease in exchange acidity of B_4 in Na_2SO_4 above $N/10$ we are unable to explain unless it can be ascribed to abnormal changes in the activity coefficients of the various ions at high concentrations in the inside solution. (An abnormal decrease in the activity coefficient of the SO_4 ions or an increase of that of the Na ions in the inside solution would account for the phenomenon.)

The influence of humus. The differences in amphoteric behavior between Las Mesas I and IV is to a large extent ascribed to humus acidoids. A systematic study of the influence of humus upon the reactions of the laterite was made by adding 2.5, 5.0 and 10.0 percent of humus acidoid (obtained by extracting peat with $NaOH$, precipitating with H_2SO_4 and electrodialysing the precipitate until free from acid) to the Las Mesas IV and then determining the pH in water and in increasing concentrations of Na_2SO_4 and $BaCl_2$. The results are shown in fig. 20.

Compared with the sample to which no humus was added we note the following:

1. The pH in water is lowered by the humus.
2. The exchange alkalinity in Na_2SO_4 is reduced and this reduction is much greater in the concentrated (N/1) solution which yields an exchange acidity with 5 and 10 percent humus. This gives rise to pronounced maxima in exchange alkalinity in the dilute solutions (between $n/100$ and $n/10$ initial concentration).
3. The exchange acidity in BaCl_2 is increased and this increase is greater in the concentrated solution (N/1) so that the maxima in dilute solutions (about N/10) become less pronounced and finally vanish (with 10 percent humus).

That this is all in agreement with the theory is best shown in fig. 21 in which the exchange alkalinities and exchange acidities are plotted against the salt concentration in analogy to fig. 9.

It will be noted that the curves in fig. 21 are »deformed» toward the lower right side. This is doubtless, in part, due to the SO_4 ions present in the laterite. The precipitation of these ions as BaSO_4 leads to an additional exchange acidity, as above explained. This causes the maximum in exchange acidity to occur at a higher concentration of salt (about N/10) than would be the case in the absence of the SO_4 ions. The »deformation» of the curves may also, in part, be an expression of the aforementioned dissolution and hydrolysis of the compound formed by the anions with the basoid groups thus changing the exchange alkalinity to an exchange acidity. In laterites having high equi-ionic points this effect is small.

It should be pointed out that since our salt concentrations express the initial (not the end) concentrations the curves do not represent the exact equilibrium conditions. The maxima occur in reality at a somewhat lower concentration than shown in the figures. Since this deviation is different for the different ions as well as for differences in the composition of the complex this will also affect the form and relative posi-

tion of the curves. A comparison of fig. 9 and 21 leaves, nevertheless, no doubt as to the application of the theory.

It should also be pointed out that the maxima in fig. 21 should, in reality, not occur at one and the same concentration of salt as in fig. 9, which is based on a single »dilute» concentration, but should be deflected as explained in connection with fig. 11 and 19. The fact that this deflection is not in evidence in fig. 20 and 21, with respect to the maxima in exchange alkalinity, leads us to add a few more words concerning this experiment.

In fig. 20 and 21 there is no reduction in the exchange alkalinity beyond the two first additions of humus (2.5 and 5 percent) and there is no visible deflection of these maxima in the direction of a lower concentration of salt.

We consider this to be significant and to be an expression of the condition of the mixture. The humus acidoid was added to the soil in the form of a gel. It seems obvious that, in spite of the shaking, the relatively large particles of the humus could not enter the smallest capillaries of the laterite but could merely interact with the basoid groups situated on the surface. Only the first additions of humus, which »neutralized» these outer groups, had, therefore, any effect on the basoid activity. The basoid groups in the interior were left free to interact with the SO_4 ions. It is obviously possible for a system to contain free acidoids and basoid groups of any strength as long as these groups are prevented from interacting. This fact may have important applications because it is theoretically possible to remove practically all the anions and cations from a solution by alternately passing it through an acidoid and a basoid.

If, in the above experiment, we had used Na-humate instead of the humus acidoid (and electrodyalysed the mixture) we would undoubtedly have obtained a different picture although the acidoid-basoid mass ratio would have been the same.

Besides the vertical series from the B horizons of the Unden podzol profile $x = 400$, we have, as already stated, selected a

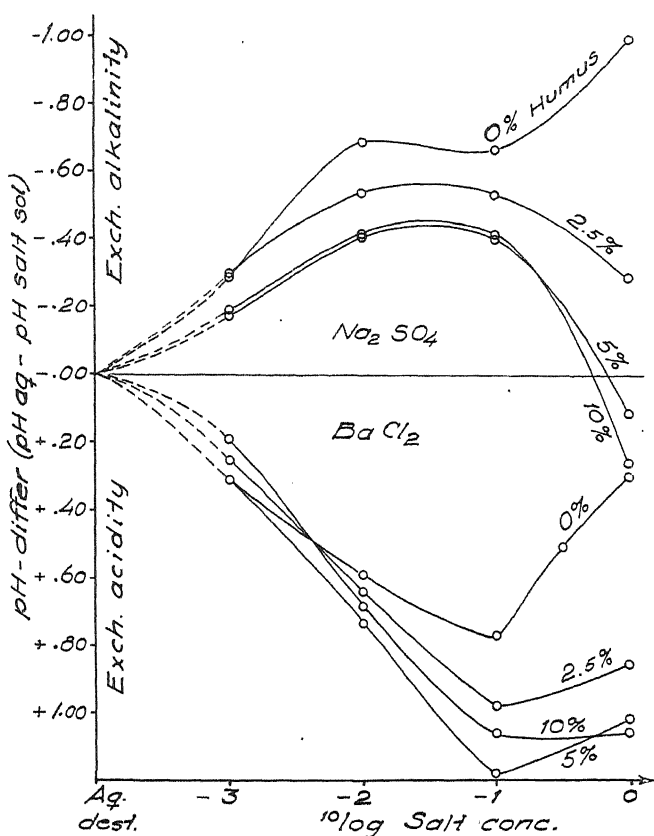


Fig. 21. The same as fig. 20, presented in analogy to fig. 9.

horizontal series by taking the most strongly basoid sample (cf. fig. 24) from three of Uden series of profiles.

Fig. 22 gives the pH in water and in different concentrations of Na₂SO₄ and BaCl₂. Sample x = 500 (y = 30) comes from the dry end of the profile series and is the most strongly basoid of all. With a loss on ignition of only 1.47 percent this samples yields an exchange alkalinity with all but the dilute solutions of BaCl₂. The nearest theoretical counterpart is found in fig. 11 d.

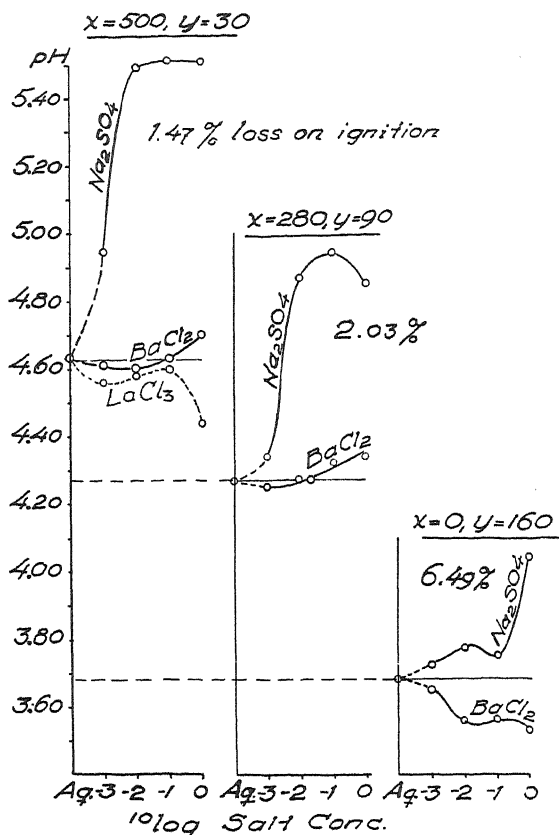


Fig. 22. The exchange reactions of the most basoid samples (cf. fig. 24) from three of the Unden profiles.

Sample $x = 280$ ($y = 90$), with its 2.03 percent loss on ignition is somewhat less strongly basoid, but even this sample yields a slight exchange alkalinity in N/1 BaCl_2 . The nearest theoretical counterpart is found in fig. 11 c.

Sample $x = 0$ ($y = 160$) with 6.49 percent loss on ignition yields curves which seem difficult to interpret. It should be pointed out that this sample comes from the wet end of the profile series and represents apparently gleyed material. The

low pH might be another factor. The sample possesses apparently a fairly strong basoid group in spite of the high humus content but before the samples have been analysed it is impossible to say what specific influence may be present.

Lanthanum. The influence of the trivalent La ion was studied in two of the Uden samples (cf. fig. 19 and 22). We note that the La ion tends to deflect the maximum in exchange acidity toward a lower concentration of salt as compared to the effect of the divalent Ba ions (cf. fig. 22). This is what the theory demands. But LaCl_3 does not yield any exchange alkalinity where BaCl_2 does (fig. 22). With a minimum in exchange acidity at $n/10$ the La yields a marked increase in exchange acidity in high concentrations, a tendency which, to a lesser degree, is also manifested by Ba in several of the samples. For this behavior we offer two explanations:

1. In high concentrations the activity coefficient of divalent and, to a still higher degree, of the trivalent ions in the micellar (inside) solution suffers an »abnormal» suppression.
2. The Ba and, to a still higher degree, the La ions displace Al ions which at low pH enter into the cationic exchange. The acidity is then developed through hydrolysis.

The influence of the concentration of the colloid.

Having shown how variations in the acidoid/basoid activity ratio affect the maxima in exchange acidity and exchange alkalinity it remains to show how variations in the concentration of the colloid affect these maxima when the acidoid/basoid ratio remains the same.

According to fig. 10 an increase in concentration should deflect the maxima toward a higher concentration of salt. Fig. 23 shows the exchange alkalinities in various concentrations of Na_2SO_4 of the B₂ sample of the Uden profile 400 in the proportions of 6, 12 and 20 grams of soil to 20 cc. solution.

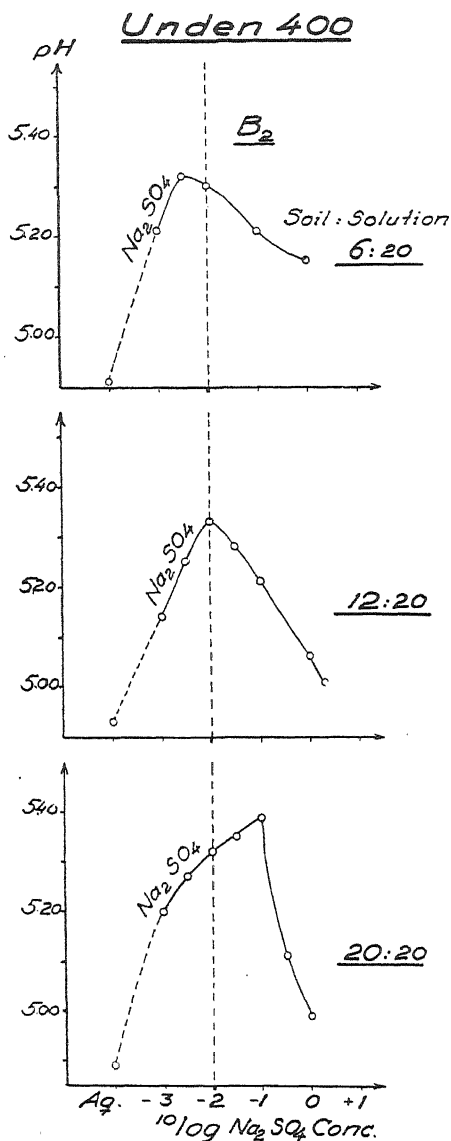


Fig. 23. The influence of the concentration of the soil suspension on the maximum in exchange alkalinity.

The curves show that an increase in the concentration of the soil results in (1) an increase in exchange alkalinity and (2) a deflection of the maximum toward a higher concentration of salt.

We note further that the right legs of the curves become steeper with increasing amount of soil. This is because the counter action (exchange acidity) of the Na ions is less effective in the dilute suspension as a result of dilution which opposes the lowering and favors the elevation of the pH.

The maximum in exchange alkalinity in a dilute suspension should be smaller than the exchange alkalinity of a more concentrated suspension at the corresponding concentration of the salt solution. This is true for the 12:20—20:20 pair but, for some reason, not for the 6:20—12:20 pair of systems.

With this exception

the results are in perfect agreement with the theory and yield information which, if it could be quantitatively interpreted, would be of very great importance, for we should then be able to determine the quantity of colloid in a soil merely by means of a few simple pH determinations. But even the qualitative results which the method yields will lend themselves to important applications.

Application to soil profiles.

Having established the factors which govern the amphoteric reactions of soils we are in a position to reciprocate and by means of these reactions, i. e., the pH, determine qualitatively and, in a comparative sense, quantitatively, these factors.

We are here presenting the results of the application of the method to three of the Uden profiles and to the Las Mesas laterite.

Fig. 24 gives the difference between the pH of the soil in water and its pH in $N/100 \text{ Na}_2\text{SO}_4$ and BaCl_2 solutions at different depths of the profiles. These differences express the exchange acidity (positive values) and exchange alkalinity (negative values) of the soil materials.

Since the samples have not yet been analysed it is too early to attempt a detailed discussion of the results. In the case of the podzol profiles we shall merely call the readers attention to the maximum in exchange alkalinity in the upper B horizon. This maximum expresses a high basoid activity resulting from the isoelectric precipitation of the cationic sol complex which has come from the A horizon. Then there is another maximum in the gley horizon (G) due to accumulation of ferric hydroxide formed by the oxidation of soluble ferrous compounds.

This method which is based upon a determination of the *activities* of the acidoid and basoid groups (as distinguished from the analytical determination of the *quantities* of acidoid and basoid materials) offers a simple, direct and scientific ex-

pression for the podzolization of a soil. (For a complete report on the work on the Uden series cf. MATTSON and LÖNNEMARK).

The samples of the laterite used in this experiment were electro dialysed to remove the displaceable SO_4 ions which were present in small but considerable amounts in the II, III and IV samples. Sample I contained very little SO_4 but contained, on the other hand, appreciable quantities of Ca ions of which the other samples contained mere traces.

The pH of the electro dialysed samples (the ultimate pH) in water and in N/100 and N/1 Na_2SO_4 and BaCl_2 are given in table 6. These values represent the average of duplicate determinations with the glass electrode which gave very good results. On the basis of these values the basoid activity is greatest in sample II whereas the acidoid activity is greatest in sample I, which contains considerable humus acidoids.

The differences between the pH values in water and in the salt solutions are shown graphically in fig. 24. We get here a different picture from that of the podzol profiles. The characteristic maxima and minima in the podzols are absent in the laterite. The exchange alkalinity of the laterite in the sulphate solutions is lowest in the surface soil and attains a uniform high value in all the subsoil samples. The exchange acidity in BaCl_2 ought to be greater in sample I than in the three subsoil samples. The fact that this is not the case must be ascribed to the SO_4 ions which, in spite of a prolonged electro dialysis (one week), still remained in samples II, III and IV, as was shown by extracting the soil with a hot ammoniacal solution of NH_4Cl . Since these subsoil samples yielded a still greater exchange acidity before being electro dialysed we conclude that the exchange acidities of these samples are still »abnormally» high whereas the exchange acidity of sample I more nearly expresses the true amphoteric nature of the soil complex.

Another significant thing in fig. 24 (cf. Las Mesas) is the fact that the exchange alkalinity is greater in N/1 than in N/100 NaSO_4 and that the exchange acidity is greater in N/100

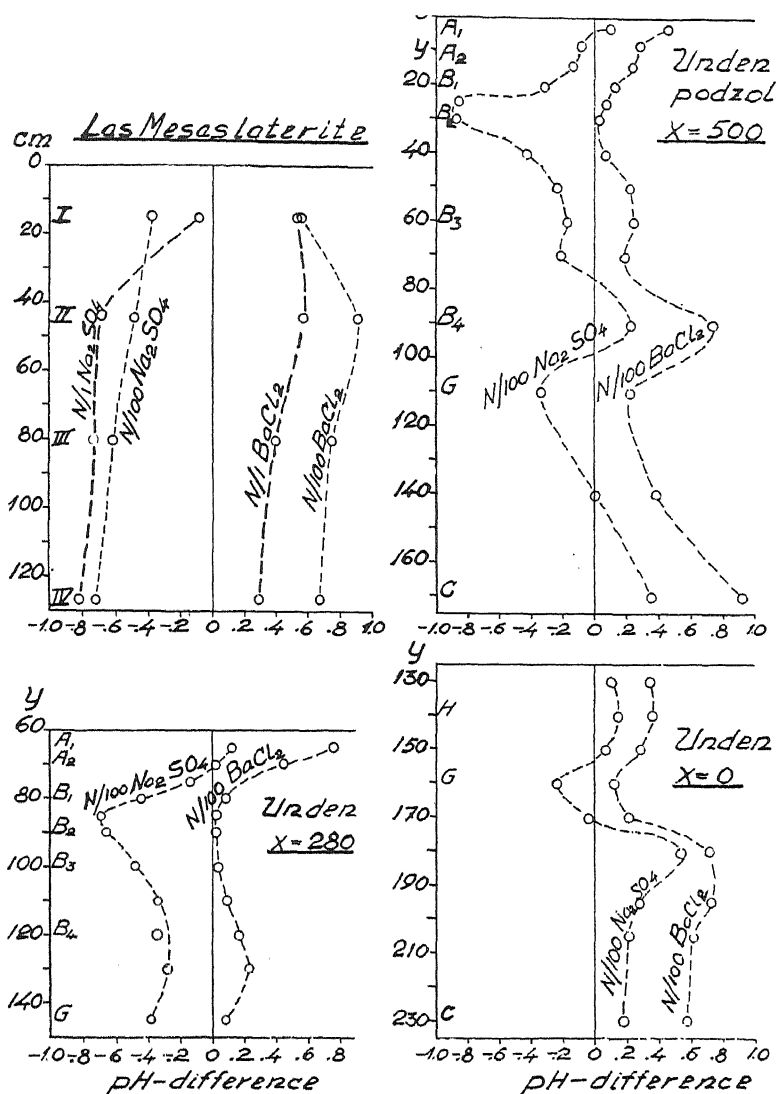


Fig. 24. The difference between the pH in water and in the salt solutions of the Las Mesas laterite and of three of the Uden podzol profiles. Negative values = exchanges alkalinity. Positive values = exchange acidity.

Table 6.

The loss on ignition and the pH of the electrodialysed Las Mesas laterite in water and in salt solutions.

Sample	Depth in inches	Percent loss on ignition	pH in:				
			water	N/100 Na ₂ SO ₄	N/1 Na ₂ SO ₄	N/100 BaCl ₂	N/1 BaCl ₂
I	0—12	18.53	4.25	4.63	4.33	3.70	3.72
II	12—23	16.52	6.11	6.60	6.79	5.20	5.54
III	26—38	15.89	5.60	6.21	6.33	4.85	5.20
IV	43—58	16.75	5.79	6.51	6.61	5.12	5.50

than in N/1 BaCl₂ in samples II, III and IV. This tells us that the basoid groups are very strong in these samples; so strong that (1) the effect of the SO₄ ions *remains* stronger than that of the Na ions even in high concentrations and that (2) the effect of the Cl ions *becomes*, in high concentrations, greater than that of the Ba ions. In sample I the acidoid group is sufficiently strong to make the effect of the Na ions outweigh that of the SO₄ ions in high concentrations. The same tendency is shown with respect to the Ba and Cl ions.

Other applications.

It has been pointed out that theoretically it would be possible to remove practically all the anions and cations from a solution by passing it through alternate layers, or a coarse mixture, of acidoids and basoids. In order to find out to what extent this is possible in the case of soil acidoids and basoids we performed the following experiment:

Twenty grams of electrodialysed raw humus and fifty grams of Las Mesas II were separately moistened (to prevent intimate mixing and mutual interaction) with N/100 CaSO₄ and then lightly mixed and placed in a tall glass funnel. The mixture was then leached with the CaSO₄ solution with the result that

neither the first, second, third or fourth 50 cc of the filtrate showed any trace of Ca or SO_4 ions. A special study of this problem is planned.

The Donnan distribution of ions in the soil has many other important consequences. Consider, for example, the effect of a concentration and a dilution of the soil solution on the mobility of the mono and divalent ions. A dilution favors the ingo (in the soil complex and in the roots of plants) of the divalent and the outgo (in the soil solution) of the monovalent ions. A concentration reverses the process. One might say that acidoids and basoids »inhale» divalent and »exhale» monovalent cations and anions, respectively, upon wetting and »inhale» monovalent and »exhale» divalent cations and anions upon drying.

The »*dilution effect*» on alkali soils observed by EATON and SOKOLOFF (1935), and by KELLEY (1939) is obviously an expression of such an ionic »respiration»; of a readjustment of an equilibrium, between ions of different valence, which has been disturbed through a change in concentration in the outside solution. Eaton and Sokoloff found that: »*The amount of sodium, both relative and absolute, in the aqueous phase tends to increase as the water-soil ratio is increased, whereas the relative concentration of calcium (and magnesium in some cases) tends to decrease. The absolute amount of calcium in solution may remain unchanged or tend to increase with dilution when soils contain an excess of sparingly soluble salts such as CaCO_3 and CaSO_4 .*»

This » *CaCO_3 effect*», which tends to obscure the valence effect, is very significant from the point of view of reclamation of calcareous alkali soils. In a noncalcareous alkali soil the dilution (or leaching) effect would not be very great because the mobile Ca ions would soon be exhausted. In a calcareous soil the Ca reserve is great and since »the energy of adsorption» of the Ca ions is greater than that of the Na ions (*in dilute solution*), the low solubility of CaCO_3 is here an obvious asset: it allows the soil complex to take a »deep and prolonged

breath» of Ca ions when the soil is leached, even when the irrigation water contains a few milliequivalents of alkali salts per liter.

Since a leached soil means a dilute soil solution we can readily understand why, in the humid soils, the divalent base forming cations are present in the exchange complex in a greater proportion than in the original silicate rocks and in the soil solution.

As to the dilution effect on the anions, that will depend on the amphoteric nature of the soil and on the pH at which the analysis is made. High above the equi-ionic point the anions are negatively adsorbed ($y < x$ in equations 11 and 12). At lower pH at which the basoids are in function the anions are positively adsorbed ($y + z > x$) and the dilution effect, with respect to the valence, will be the same as for the cations at high pH, provided there is no hydrolysis and no solution effect (CaSO_4 etc.). The investigator must know the amphoteric character of his soil, its equi-ionic point, the pH at which he is working, the volume of the micellar solution and many other things before he can, even qualitatively, interpret his results.

Conclusion.

The dilution effect leads Kelley to the noteworthy conclusion that »there is no such thing as a definite or true soil solution». This statement emphasizes the dynamic character of the soil which is subject to constant changes in response to its environment. The truth of the statement is best realized when we consider that hand in hand with the redistribution of the ions, resulting from changes in concentration, there are changes in P.D., changes in the osmotic and electrical transfer of water (swelling and shrinking), changes in dispersion and Brownian mobility and many other changes. The amphoteric character of the complex and the conception of an amphoteric ion atmosphere make the picture more complex. Clusters or »clouds» of ions of opposite sign of charge, Brownian bombardments,

charges and discharges must give rise to a turbulence comparable to the electrical storms in our terrestrial atmosphere.

There is perhaps nothing which better testifies to the inconstancy of soil factors than the pH and the capacity to bind base. Kelley might have included in his statement that *there is no such thing as a definite or true soil pH nor any definite or true soil capacity to bind base (or acid)*. Apart from the changes in the activity coefficients with changes in the ionic strength of a solution, common to all electrolytes, we meet, in the case of colloidal electrolytes, with the peculiar conditions of an unequal distribution of ions. This distribution is affected by salts in such a way that the pH of a soil can be both higher and lower in solutions of different concentrations of the same salt than the pH of the soil in water. As to the capacity to bind base at a given pH we know from our titration curves that this capacity will be different in different solutions. Above the point of exchange neutrality of two concentrations the capacity will be greater in the stronger solution, whereas below this point it will be greater in the dilute solution.

Since the pH of a given soil at a given base status is a function of the concentration and composition of the soil solution and since the net capacity of the soil to bind base at a given pH is an expression of the power of the base forming cations to displace the H ions of the acidoids, over and above the power of the acid anions to displace the OH ions of the basoids and further, since the relative displacing power of ions of different valence changes with changes in concentration, we arrive at the conclusion that *the pH, the concentration and composition of the soil solution and the capacity of the soil to bind base at a given pH are interdependent variables*. This conclusion may be summed up in the following statement: *At each pH of the soil there is a different soil solution, a different pH and a different capacity to bind base*. The soil problems are complicated, but it has been said that *»it is easier to find your way in a natural cave than in a man-made labyrinth»* and the soil is a natural body.

Summary.

The property of amphoteric soils simultaneously to exchange H and OH ions for the cations and anions of a neutral salt solution at, or near the equi-ionic point of the soil, and the valence effect in the mass law (as expressed by the Donnan distribution of ions between the soil complex and the soil solution) lead to some very significant, but heretofore neglected, consequences.

For the salts Na_2SO_4 and CaCl_2 the following expressions apply:

for acidoids:

$$\frac{(\text{H}')_i}{(\text{H}')_o} = \frac{(\text{Na}')_i}{(\text{Na}')_o} = \frac{V(\text{Ca}'')_i}{V(\text{Ca}'')_o}$$

and for basoids:

$$\frac{(\text{OH}')_i}{(\text{OH}')_o} = \frac{(\text{Cl}')_i}{(\text{Cl}')_o} = \frac{V(\text{SO}_4'')_i}{V(\text{SO}_4'')_o}$$

where the parenthesis represents activity and i and o signify that the ions are in the inside and the outside solutions respectively.

The fact that the divalent ions enter the equation in the form of the square root of their activity tells us that, in low concentrations (in the »outside» solution) of the salt and in the presence of equivalent proportions of free acidoids and basoids, the Ca ions will displace more H ions than the Cl ions will displace OH ions, and that the SO_4 ions will displace more OH ions than the Na ions will displace H ions. In high concentrations the displacing power of the different ions will approach the same value. This is all provided that none of the ions enter into any specific reaction.

From this it follows that, under the above conditions, CaCl_2 (or a salt of the type $\text{M}'\text{S}'_2$) must yield a maximum in exchange acidity in dilute solutions and that Na_2SO_4 (or a

salt of the type M_2S'') must yield a maximum in exchange alkalinity in dilute solutions.

A theoretical study has been made of the various factors which determine the position and magnitude of these maxima such as the acidoid: basoid ratio, the concentration of colloid and the concentration of the ions in the micellar solution.

By means of titrations and pH determinations of different soils in various concentrations of salts it has been possible to prove the application of the theory.

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The decomposition of the organic compounds in barnyard manure.

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As regards the decomposition of barnyard manure in arable soil the mineralization of the protein nitrogen is of the greatest interest from a practical point of view. We have studied this problem in a long series of investigations (BARTHEL 1917, BARTHEL & BENGTTSSON 1918, 1920, 1924, 1926, 1930, 1931 *a*, 1931 *b*, 1934, 1939, BENGTTSSON 1932, BENGTTSSON & BARTHEL 1935). This mineralization represents, however, only a part of a much more complicated problem which includes besides the decomposition of the proteins, also the decomposition of the other organic components of the manure, e. g. the cellulose, the pentosans and the lignin. We have now extended our investigations of manure to include a study of the decomposition of these three compounds and also determinations of the α -humus and of the organic carbon.

Scheme of experiments.

The experiments were made in sand at the initial pH-values 8 (samples I—III) and 6 (samples IV—VIII). In the former case the sand had been washed only with water, in the latter, with hydrochloric acid and water. This latter washing was carried out in order to reduce the rather high content of lime and thus to facilitate the adjustment of the lower pH-value.

On the basis of 100 g. dry sand the nitrogen contents were 1.6 mg. and 0.7 mg., respectively. The reason why sand poor in nitrogen instead of soil was used for the experiments was that an earlier investigation had shown a highly irregular mobilization of the soil nitrogen; with such material it is naturally impossible to establish the degree of nitrogen mineralization in the manure added (BENGTSSON & BARTHEL 1935).

The manure used was obtained from cows which had been fed on hay, straw and concentrates; it was three months old and contained urine, straw and chaff. After thoroughly mixing and grinding of the manure the following figures were obtained:

Water	78.95	%
Ash	3.78	»
Ammonia-N	0.0856	»
Soluble N ¹	0.1029	»
Nitrate-N	0	
Total-N	0.476	»
Protein-N ²	0.390	»
Lignin ³	3.58	»
Pentosans	3.84	»
α -Humus	1.19	»
Cellulose	5.37	»
Organic carbon	8.50	»

The nitrogen figures for the manure were established in the moist manure just before the start of the experiments. The other determinations were made at the same time on a sample of the manure dried at 90° C. and then ground in a Wiley mill (1 mm sieve); this sample contained 5.83 per cent water.

All the samples of the experiments contained 7560 g. dry sand, 840 g. ground manure and 4.2 g. KH_2PO_4 ; they were inoculated with 120 ml. soil extract which was prepared from

¹ Determined according to the method of *Glathe* (1927).

² Assumed to be equal to the difference between total-N and ammonia-N values.

³ The percentage figure represents lignothioglycolic acid.

two clays with ordinary humus content and with the pH-values 5.0 and 7.5, respectively. In addition the samples received:

Sample	I.	No addition							
»	II.	6.06 g. KNO_3							
»	III.	12.12 »	»						
»	IV.	252 ml. 1 N. H_2SO_4							
»	V.	3.97 g. $(\text{NH}_4)_2\text{SO}_4$	plus	252 ml. 1 N. H_2SO_4					
»	VI.	7.94 »	»	»	»	»	»	»	»
»	VII.	6.06 »	KNO_3	»	»	»	»	»	»
»	VIII.	12.12 »	»	»	»	»	»	»	»

The amount of sulfuric acid added to samples IV—VIII adjusted the acidity to a pH-value 6, as shown by separate experiments. The addition of potassium nitrate and ammonium sulfate, which corresponded approximately to one or two equivalents of the ammonia nitrogen in the manure, were made in order to establish whether the processes of decomposition studied could be accelerated. It may be mentioned that these experiments were started before the similar investigations on the decomposition of barley stubbles and roots had been finished (BENGTSSON 1936 *a*). To the alkaline samples II and III potassium nitrate was added; additions of ammonium sulfate were not made to alkaline samples because there was thought to be a risk that a part of the ammonia added could evaporate. To the acid samples V—VIII either potassium nitrate or ammonium sulfate was added, in order to establish whether the decomposing microorganisms would prefer the nitrate nitrogen, as was indicated by an earlier investigation on cellulose decomposition (ANDERSON 1925). The water content was adjusted to 15 per cent in all the samples. After thorough mixing, each sample was divided into 21 parts, which were put into 300 ml. glass jars. The jars were stoppered in a manner previously described (BARTHEL & BENGTSSON 1939). After weighing, the jars were incubated at 20—22° C. When necessary, water was added to replace

losses by evaporation. As both the sand washed with hydrochloric acid plus water and that washed with only water contained small amounts of organic material, it was thought necessary to make up two check samples with the initial pH-values 8 and 6. These samples contained 7560 g. dry sand, 4.2 g. KH_2PO_4 , 120 ml. soil extract and 6.06 g. KNO_3 but no manure; the water content was 11 per cent. They were put into 21 glass jars, which were stoppered and incubated as mentioned above. The checks were analyzed for α -humus, lignin and organic carbon at the same times as the other samples; the values found were assumed to allow the correction of the values for α -humus, lignin and organic carbon in the manure added to samples I—VIII. No corrections were necessary for cellulose and pentosans as the sand was free from these compounds.

Analytical methods.

After $\frac{1}{4}$, 1, 2, 3 and 4 years two jars of each kind were analyzed in the following way:

pH was determined according to the quinhydrone method.

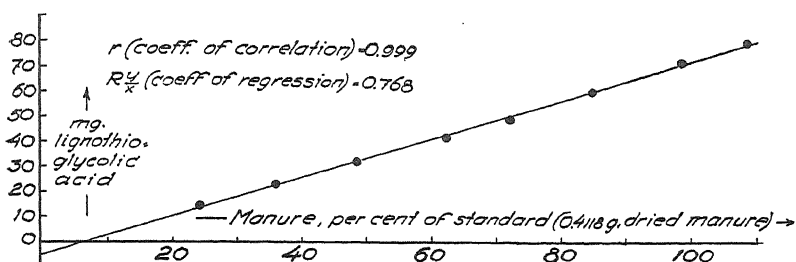
Total-N was determined according to the method of DAVISSON & PARSONS (1919), slightly modified by us, on two 40 g. samples from each of the two jars.

Ammonia-N was determined in 200 ml. extract, prepared from 100 g. sample and 250 ml. 0.5 N. KCl, by distillation with MgO (BENGSSON 1924).

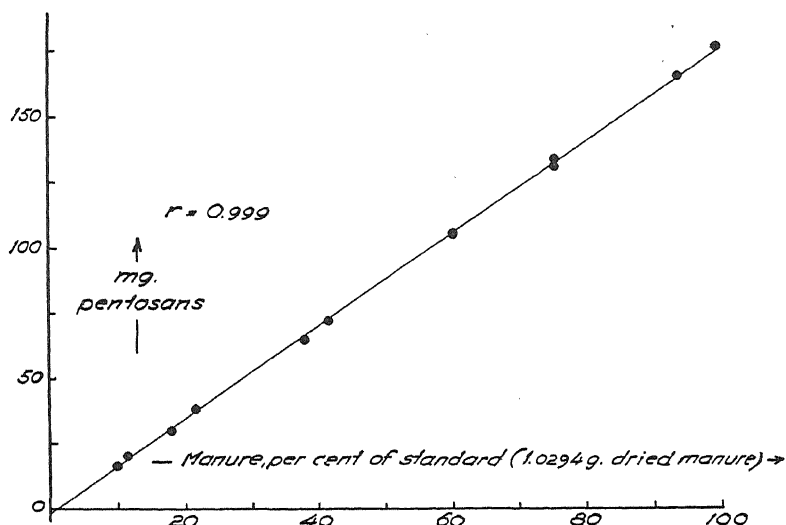
Nitrate-N was determined in the residue from the ammonia distillation according to a modified Devarda method (BENGSSON 1932).

Cellulose was determined on 40 g. samples according to the method proposed by BENGSSON (1925).

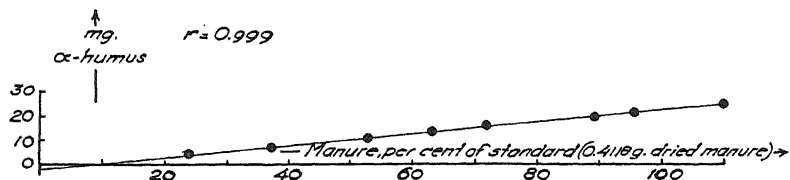
Pentosans were determined on 50 g. samples according to the method of KULLGREN & TYDÉN (1929); the samples were first freed from nitrites and nitrates by washing with water (BENGSSON 1936 b).



Diagr. 1. The relation between the yield of lignothioglycolic acid and the amount of manure. Experiments made in sand washed with hydrochloric acid plus water. (Sambandet mellan utbytet av lignotiolglykolsyra och provets halt av gödsel; försöket utfört i sand, tvättad med saltsyra och vatten).

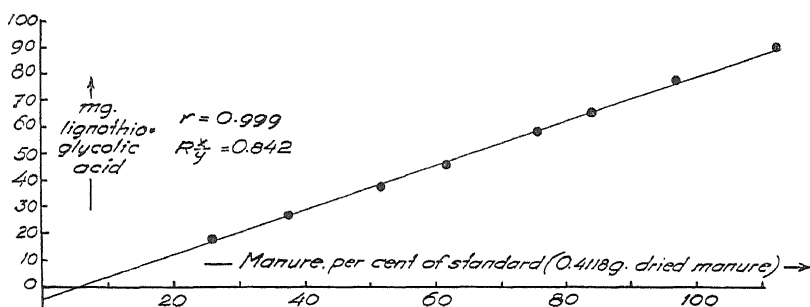


Diagr. 2. The relation between the yield of pentosans and the amount of manure. (Sambandet mellan utbytet av pentosaner och provets halt av gödsel.)



Diagr. 3. The relation between the yield of α -humus and the amount of manure. (Sambandet mellan utbytet av α -humus och provets halt av gödsel.)

Lignin was determined on 20 g. samples according to Holmberg's thioglycolic acid method, as modified here (HOLMBERG 1930, 1934, 1936, BENGTSSON 1936 *a*). By means of this method the lignin is transformed into lignothioglycolic acid. Earlier investigations having shown that samples containing proteins will give lignothioglycolic acid preparations which contain nitrogen (BENGTSSON 1936 *a*, 1938), we have now considered it necessary to determine this nitrogen content;



Diagr. 4. The relation between the yield of lignothioglycolic acid and the amount of manure. Experiments made in sand washed only with water. (Sambandet mellan utbytet av lignotioglykolsyra och provets halt av gödsel; försöket utfört i sand, tvättad endast med vatten.)

however on the samples taken after 3 months no determinations were made. The nitrogen determinations were made on lignothioglycolic acid preparations from duplicate 20 g. samples.

Organic C was determined on 20 g. samples according to the method of SIMON & GUILLAUMIN-BALKS (1923, 1925); the carbon dioxide obtained was determined gravimetrically.

α -Humus was determined on 20 g. samples according to WAKSMAN'S method (1926), somewhat modified by us; only one extraction with alkali was made.

The methods for the determination of lignin, pentosans and α -humus were controlled on increasing amounts of manure mixed with dry sand. For these controls dried manure with 5.88 per cent water was used (p. 56); in the case of the determination of pentosans the amount of sand used was 41.5 g., in the two other cases 16.6 g. sand were used. The results

are presented in diagrams 1—3, where the ordinates represent mg. of lignothioglycolic acid, pentosans and α -humus, determined respectively and the abscissas the amounts of manure used, expressed in per cent of the standard amounts of dried manure. The diagrams show that the three functions studied are linear. The experiments just mentioned were made with sand washed with hydrochloric acid plus water. With sand washed only with water very nearly the same diagrams for pentosans and α -humus were obtained whereas the lignin determinations with this sand gave a diagram (diagr. 4) with a considerable larger $R_{\frac{y}{x}}$ value than that of diagr. 1. The fact that sand can influence the yield of lignothioglycolic acid has been found in an earlier investigation (BENGTSSON 1936 *a*).

Results.

The initial values are reported in table 1 and the results after $\frac{1}{4}$, 1, 2, 3 and 4 years in tables 2—6. The figures for total nitrogen are based upon the results from four determinations, the other figures from two determinations. In tables 2—6 the values for total nitrogen, ammonia nitrogen, nitrate nitrogen and α -humus are reported on the basis of 100 g. samples. The figures for lignin decomposed are established with the aid of the amounts of lignothioglycolic acid found in 20 g. samples and diagrams 4 and 1 for the samples I—III and IV—VIII, respectively. The calculation of the figures for protein decomposed may be illustrated by an example. Sample I had lost 4.7 mg. total nitrogen during the first year (table 1) and the amount of ammonia nitrogen plus nitrate nitrogen had increased from 7.9 to 9.1 mg. during the same time (tables 1 and 3). Thus, the protein nitrogen decomposed during this time was assumed to be $(4.7 + 9.1 - 7.9 =) 5.9$ mg., corresponding to 16 per cent of 35.9 (table 1).

The experiments show the following:

All the samples had lost nitrogen, though the losses were rather small for the acid samples to which potassium nitrate

Table 1.

Initial values on the basis of 100 g. samples.

Samples	pH	Total-N ¹ mg.	Ammonia-N mg.	Nitrate-N mg.	Protein-N mg.	α -Humus mg.	Cellulose mg.	Pentosans mg.	Lignin ² mg.	N-content of the lignothioglycolic acid, per cent	Org. C mg.	C : N ³
I	8.1	45.1	7.9	0.0	35.9	110	494	354	388	2.9	783	99
II	8.1	54.4	7.9	9.2	35.9	110	494	354	388	2.9	783	46
III	8.1	63.6	7.9	18.4	35.9	110	494	354	388	2.9	783	30
IV	6.1	44.4	7.9	0.0	35.9	110	494	354	357	2.5	783	99
V	6.1	53.7	17.1	0.0	35.9	110	494	354	357	2.5	783	46
VI	6.1	62.9	26.3	0.0	35.9	110	494	354	357	2.5	783	30
VII	6.0	53.7	7.9	9.2	35.9	110	494	354	357	2.5	783	46
VIII	5.7	62.9	7.9	18.4	35.9	110	494	354	357	2.5	783	30

had not been added (samples IV—VI). For each sample the losses were of about the same magnitude after 1, 2, 3 and 4 years as after 3 months, indicating that the losses had occurred mainly during the first 3 months, when the decomposition of the cellulose and the pentosans was taking place most rapidly. Both at the alkaline and the acid reaction the losses were larger for the samples to which potassium nitrate had been

¹ In all the tables the total-N of the sand, 1.3 mg. for samples I—III and 0.6 mg. for samples IV—VIII, is included.

² The figures represent mg. lignothioglycolic acid. In a preliminary report recently left to the third Commission of the International Society of Soil Science we have given the figure 330 for all the samples. This value was found in determinations made on manure without sand. We have now considered it more accurate to give both the figures determined for manure mixed with sand which had been washed with water only and those obtained when the sand admixed had been washed with hydrochloric acid plus water.

³ The figures represent the ratio of organic carbon to ammonia-N plus nitrate-N.

Table 2.
Results after 3 months.

Samples	pH	Total-N mg.	Loss of total-N mg.	Ammonia-N mg.	Nitrate-N mg.	α -Humus mg.	Per cent decomposed				
							Cellulose	Pentosans	Lignin	Org. C	Protein-N
I	8.0	40.5	4.6	0.3	1.3	84	84	73	18	34	— 5
II	8.1	45.7	8.7	0.1	4.5	85	86	75	16	36	— 11
III	8.1	54.7	8.9	0.1	15.5	81	86	76	20	37	— 5
IV	6.0	42.4	2.0	2.2	2.2	86	69	56	5	22	— 4
V	5.9	51.9	1.8	12.1	1.7	83	66	54	0	23	— 4
VI	5.9	60.2	2.7	20.4	1.1	74	67	55	4	27	— 6
VII	5.8	47.7	6.0	0.3	9.9	87	66	—	1	25	— 3
VIII	5.8	55.2	7.7	0.3	17.6	80	62	—	1	25	— 2

Table 3.
Results after 1 year.

Samples	pH	Total-N mg.	Loss of total-N mg.	Ammonia-N mg.	Nitrate-N mg.	α -Humus mg.	N-content of the lignothiolglycolic acid, per cent	Per cent decomposed				
								Cellulose	Pentosans	Lignin	Org. C	Protein-N
I	7.5	40.4	4.7	0.1	9.0	89	3.6	94	81	38	53	16
II	7.7	46.1	8.3	0.0	13.1	86	3.2	94	84	32	55	12
III	7.6	56.7	6.9	0.1	23.5	92	3.2	95	83	36	55	12
IV	5.7	42.4	2.0	3.2	5.8	105	2.9	77	63	26	41	9
V	5.6	52.4	1.3	11.4	5.8	102	3.0	73	61	17	36	4
VI	5.5	59.9	3.0	19.3	5.7	107	3.1	75	59	14	36	5
VII	5.5	47.9	5.8	2.1	13.5	102	3.1	75	61	17	40	12
VIII	5.5	56.0	6.9	0.9	20.6	102	3.3	79	61	13	28	6

Table 4.
Results after 2 years.

Samples	pH	Total-N mg.	Loss of total-N mg.	Ammonia-N mg.	Nitrate-N mg.	α -Humus mg.	N-content of the lignothioglycolic acid, per cent	Cellulose	Pentosans	Lignin	Org. C	Protein-N
								Per cent decomposed				
I	7.4	40.1	5.0	0.2	13.1	80	3.4	95	87	43	58	29
II	7.6	44.8	9.6	0.2	17.9	83	3.6	97	88	45	59	30
III	7.5	54.2	9.4	0.1	26.8	78	3.7	97	88	46	58	28
IV	5.6	41.1	3.3	3.3	8.8	98	3.0	77	67	23	37	21
V	5.6	50.5	3.2	10.8	7.9	86	3.3	75	65	20	37	13
VI	5.5	60.3	2.6	19.9	8.3	101	3.2	74	65	24	37	13
VII	5.5	49.2	4.5	1.5	15.8	98	3.1	76	66	24	37	13
VIII	5.6	55.4	7.5	1.0	23.5	97	3.3	75	63	20	31	16

Table 5.
Results after 3 years.

Samples	pH	Total-N mg.	Loss of total-N mg.	Ammonia-N mg.	Nitrate-N mg.	α -Humus mg.	N-content of the lignothioglycolic acid, per cent	Cellulose	Pentosans	Lignin	Org. C	Protein-N
								Per cent decomposed				
I	7.1	39.9	5.2	0.1	17.3	107	3.5	97	90	52	67	41
II	7.3	44.6	9.8	0.1	22.0	93	3.5	98	91	54	67	41
III	7.2	53.5	10.1	0.1	31.9	89	3.4	98	93	56	69	44
IV	5.3	43.7	0.7	2.4	12.3	116	2.8	84	69	23	40	21
V	5.4	52.4	1.3	11.7	10.5	106	3.0	81	70	21	39	18
VI	5.2	60.4	2.5	18.7	11.5	120	3.0	78	68	11	36	18
VII	5.3	47.3	6.4	0.5	19.1	114	3.0	78	69	23	41	25
VIII	5.4	56.0	6.9	1.2	25.7	114	3.1	79	69	22	42	21

Table 6.
Results after 4 years.

Samples	pH	Total-N mg.	Loss of total-N mg.	Ammonia-N mg.	Nitrate-N mg.	α -Humus mg.	N-content of the lignothiolglycolic acid, per cent	Cellulose	Pentosans	Lignin	Org. C	Protein-N
								Per cent decomposed				
I	6.9	40.9	4.2	0.0	19.5	79	3.6	99	91	60	70	44
II	7.1	44.3	10.1	0.0	23.8	84	3.4	100	92	61	71	47
III	7.0	56.2	7.4	0.1	34.5	81	3.9	100	92	63	71	44
IV	5.1	43.3	1.1	1.5	13.6	116	2.8	86	68	22	46	23
V	5.1	52.9	0.8	10.5	12.0	91	3.0	83	69	26	45	17
VI	5.0	62.3	0.6	18.3	12.7	127	3.2	82	70	25	44	15
VII	5.1	47.9	5.8	0.9	19.7	113	2.9	86	71	29	49	26
VIII	5.3	56.4	6.5	2.9	26.9	108	2.9	89	76	37	50	28

added than for the others; thus, at least a part of the losses for the former samples must be ascribed to denitrification.

In the four years during which the material was under observation, the alkaline samples produced 14.6—19.5 mg. nitrate nitrogen per 100 g.; the corresponding limits for the acid samples were 8.5 and 13.6. At the end of this time the pH values of all the samples had been reduced by about one unit. It is remarkable that, even after four years, samples V and VI contained large amounts of ammonia nitrogen; the low pH value (5.1) possibly explains why this nitrogen has not been nitrified.

The cellulose was decomposed more rapidly than the other compounds studied. After three months actually about 85 per cent had been decomposed in the alkaline samples and 66 per cent in the acid samples. Decomposition proceeded in a parallel manner in both acid and alkaline samples. The additions of potassium nitrate and of ammonium sulfate, by which the ratio of organic carbon to assimilable nitrogen was reduced

from 99 to 46 or 30, have shown no stimulating effect upon the rapidity of decomposition of cellulose or of the other substances studied.

The pentosans were decomposed somewhat more slowly and not so completely as the cellulose. After three months about 75 per cent was decomposed in the alkaline samples and 55 per cent in the acid samples; after four years the figures were 92 and 71, respectively. The figures after three years were very nearly the same as those after four years; thus, the process was dormant during the fourth year.

On an average 18 per cent of the lignin had been decomposed in the alkaline samples after three months but only 2 per cent in the acid samples; at the end of the experiments the figures were 61 and 28, respectively. Thus, this decomposition had proceeded considerably more slowly than that of the cellulose or of the pentosans. In the acid samples the process was dormant during the two last years. It must be acknowledged that the lignin figures presented are not quite accurate, mainly on account of the fact that the lignothioglycolic acid preparations contained nitrogen. For the preparations obtained after 1, 2, 3 and 4 years the nitrogen figures were about 0.5 units lower than those for the corresponding preparations at the beginning of the experiment. This indicates that the content of crude protein in the preparations was lower at the start than at the time of the later determinations. Consequently, the values presented for decomposed lignin are probably somewhat too small.

The values found in the determinations of α -humus are rather irregular; therefore they allow no sure conclusions.

In all the samples the amount of protein synthesized after three months was larger than that of protein decomposed, as seen by the negative figures for protein nitrogen decomposed (table 2). At the subsequent times of sampling positive values were obtained. After three years an average of 42 per cent of the protein nitrogen was decomposed in the alkaline samples and 21 per cent in the acid samples; at the end of the experi-

ments the figures were 45 and 22, respectively. Thus, the process was very nearly dormant during the last year. Both after three and four years the degree of protein decomposition in samples V and VI, to which ammonium sulfate had been added, was somewhat lower than in the other acid samples; the high content of ammonia nitrogen in the former samples has probably caused this retardation.

Like the decomposition of cellulose and of the pentosans, the decomposition of organic carbon proceeded most rapidly during the first three months. After these three months an average of 37 per cent of the carbon was decomposed in the alkaline samples and 24 per cent in the acid samples; at the end of the experiments the figures were 71 and 47, respectively.

Summary.

An experimental study of the decomposition of protein, cellulose, pentosans and lignin in manure has been carried out for a period of four years. The manure was obtained from cows which had been fed on hay, straw and concentrates; it was three months old and contained urine, straw and chaff. The experiments were made in sand at the initial pH-values 8 and 6. By addition of potassium nitrate or ammonium sulfate the ratio of organic carbon to assimilable nitrogen was narrowed from 99 to 46 or 30. The following results should be noted:

All the processes studied proceeded decidedly more rapidly at the alkaline than at the acid reaction. After four years 100 per cent of the cellulose, 92 per cent of the pentosans, 61 per cent of the lignin and 45 per cent of the proteins were decomposed in the alkaline samples; for the acid samples the figures were 85, 71, 28 and 22, respectively.

The additions of ammonia nitrogen and nitrate nitrogen showed no stimulating influence upon the rapidity of the processes of decomposition. On the contrary, a slight retardation of the decomposition of proteins was found in the samples to which ammonia nitrogen had been added.

Sammanfattning.

Vid de här beskrivna försöken studerades sönderdelningen av protein, cellulosa, pentosaner och lignin i gödsel under fyra år. Gödseln var erhållen efter kor, som hade blivit utfodrade med hö, halm och kraftfoder; den var tre månader gammal och innehöll urin, halm och agnar. Försöken gjordes i sand vid de initiala pH-värdena 8 och 6. Genom tillsats av kaliumnitrat eller ammoniumsulfat förträngdes förhållandet mellan organiskt kol och assimilerbart kväve från 99 till 46 eller 30. Följande resultat böra noteras:

Alla de studerade processerna fortskredo betydligt hastigare vid den alkaliska reaktionen än vid den sura. Efter fyra år hade i de alkaliska proven sönderdelats 100 % av cellulosan, 92 % av pentosanerna, 61 % av ligninet och 45 % av proteinerna; för de sura proven voro de respektive siffrorna 85, 71, 28 och 22.

Tillsatserna av ammoniakkväve och nitratkväve visade ingen stimulerande verkan på sönderdelningsprocessernas hastighet. Tvärtom konstaterades en lätt retardation av proteinernas sönderdelning i de prov, till vilka ammoniakkväve hade satts.

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Vernalization of Winter Rye.

By EWERT ÅBERG.

Communication No. 7 from the Institute of Plant Husbandry.

Introduction.

During the last five years there has been much discussion about LYSENKO's theory of vernalization (1932). According to this theory winter cereal seeds which are chilled immediately after germinating and sown in the spring may give plants which will form ears and reach maturity the same year. In 1935 vernalization experiments were started at the Institute of Plant Husbandry of the Agricultural College. The Swedish variety Stålråg was used in all the experiments, of which a preliminary report will be given here.

Experiment in 1935.

Plan of experiment.

1. Seed not treated.
2. Swelling of seed at 20° C; 40 % water.
3. Vernalization of seed at 3° C; 40 % water.
4. Vernalization of seed at 3° C; 50 % water.

The seed was not disinfected. On the 3rd of May the seed for treatments 3) and 4) received water in three successive stages at 9 a.m., 2 p.m. and 8 p.m., each time one third of the total. To ensure regular absorption the material was stirred once each time water was added. The seed was kept at 20° C until the following morning when the first roots appeared. It was then placed in a dark cellar at a tem-

perature of 3°C and kept there until May 29th. Thus the seed was exposed to low temperature over a period of 25 days. The seed for treatment 2) received water on the 28th of May. The experiment was sown on sandy soil in the field, the area of each plot measuring 2.25 sq.m. ($1.5\text{ m} \times 1.5\text{ m}$) and the distance between rows 15 cm. Sprouting occurred on June 7th. The stand after treatment 4) was very uneven and not as good as the stand on the other plots (See Fig. 1). At the end of July a few ears appeared in the plots sown with vern-

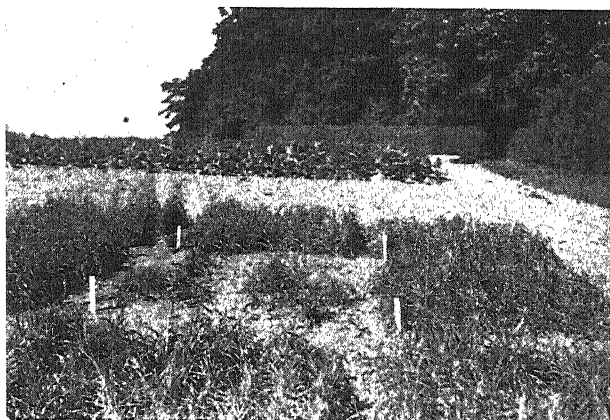


Fig. 1. Vernalization experiment with Stålråg 1935. On the right hand side are the plots with untreated seed (in the background) and with seed vernalized with 40 % water (in the foreground), and on the left hand side the plots sown with swollen seed (in the background) and seed vernalized with 50 % water (in the foreground). — Photo 10/8 1935.

alized seed, but no kernels developed. The number of ears pro plot at Aug. 23rd was for treatment 3) 14 and for 4) 5 (being the average of four replications).

Experiments in 1936.

Testing of germination and sprouting power.

The experiment in 1935 had shown that vernalization had a deleterious influence on sprouting. As this might be due to infection, further investigations were made in 1935—36

Table 1.

The effect of vernalization and disinfection of vernalized seed on germination and sprouting power 1935—36.

Water %	Disinfection mg Uspulun/g seed	Vernalization days	Germination	Abnormal plants	Sprouted plants	<i>Fusarium</i> infected plants and kernels	<i>Penicillium</i> infected plants and kernels
35	0	10	90.7	3.0	93	9	2
	0	15	87.5	8.0	84	17	5
	0	20	88.8	8.3	89	20	7
	0.2	20	89.0	4.8	91	6	21
	1.0	20	88.8	5.5	91	8	20
	0	25	86.0	6.3	86	29	16
40	0	10	89.1	4.5	90	15	1
	0	15	82.0	9.0	90	20	3
	0	20	79.3	11.0	84	37	13
	0.2	20	91.0	4.8	89	13	15
	1.0	20	90.3	3.8	97	7	21
	0	25	79.0	12.5	83	66	17
50	0	10	88.5	6.3	89	19	1
	0	15	77.3	13.5	84	20	7
	0	20	65.8	19.5	70	76	13
	0.2	20	88.8	4.8	95	17	17
	1.0	20	92.8	3.5	89	17	5
	0	25	65.3	16.8	72	81	14

with seed subjected to different treatments of disinfection and vernalization. Experiments were then made with seed not disinfected and seed disinfected with 10 and 50 % respectively of the normal dose of Uspulun powder, *i.e.* 0.2 and 1 mg of powder to each gramme of seed respectively. After being prepared in this way the seed was vernalized at 1—2° C with 35, 40 and 50 % water (Table 1). The water was given in the same way as in the 1935 experiment. Samples were taken

for the testing of germination and sprouting power¹, in the not disinfected seed after 10, 15, 20 and 25 days' vernalization and in the disinfected seed after 20 days vernalization. The results of the tests of germination and sprouting power are given in table 1. Percentages of germination and abnormal plants from untreated seed were 94 % and 3.4 % respectively, the number of plants sprouted 87 %, of *Fusarium*-infected 4 % and of *Penicillium*-infected 8 %. Following the results from the experiments in 1935 and the investigations of germination

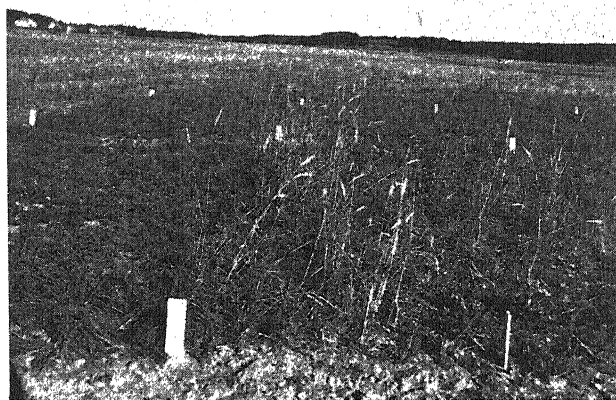


Fig. 2. Vernalization experiment with Stålråg 1936. Plot a/7. — Photo
12/9 1936.

and sprouting power 1935—36 two experiments were planned as set out (Table 2). The experiments were arranged according to the block method, with altogether 27 plots ($3 \times 3 \times 3$), the area of each plot measuring 10.5 sq.m. (1.5 m \times 7 m). Germination and sprouting power were measured in the vernalized material and the results are tabulated in table 2. Vernalization was continued until May 12th in a dark cellar at a temperature of 1—2° C. One of the experiments was sown on the same day. The seed for the other experiment was first

¹ Germination and sprouting power are determined according to the International Seed Testing methods.

Table 2.
Results of experiments in 1936.

Plot	Symbols ¹			Germination	Sprouting power		Number of ears Sept. 21st.	
	W.	D.	T.		Plants sprouted	Plants and kernels infected by <i>Fusarium</i> and <i>Penicillium</i>	Exper. 1	Exper. 2
a/1	0	0	0	81.8	91	27	—	—
a/2	0	1	1	91.3	94	17	—	1
a/3	0	2	2	90.8	94	13	—	—
a/4	1	0	1	83.8	78	33	7	4
a/5	1	1	2	88.5	95	23	34	22
a/6	1	2	0	91.0	100	9	—	—
a/7	2	0	2	82.3	64	51	120	81
a/8	2	1	0	88.9	88	23	—	3
a/9	2	2	1	92.0	91	13	1	4
b/1	0	0	1	77.0	82	27	—	—
b/2	0	1	2	89.8	89	14	—	—
b/3	0	2	0	90.0	88	15	—	—
b/4	1	0	2	81.5	81	46	71	49
b/5	1	1	0	87.9	85	35	—	—
b/6	1	2	1	94.0	89	19	3	2
b/7	2	0	0	82.0	91	25	—	—
b/8	2	1	1	93.0	84	27	4	4
b/9	2	2	2	90.0	93	18	72	32
c/1	0	0	2	79.5	90	43	—	—
c/2	0	1	0	96.8	89	22	—	1
c/3	0	2	1	93.5	93	11	1	—
c/4	1	0	0	81.5	95	30	1	—
c/5	1	1	1	91.9	92	8	20	6
c/6	1	2	2	91.8	90	18	38	47
c/7	2	0	1	77.8	85	25	11	6
c/8	2	1	2	92.7	92	31	83	30
c/9	2	2	0	92.7	89	25	—	2

¹ W₀ = Without water.W₁ = 40 % " .W₂ = 50 % " .T₀ = Without chilling.T₁ = 10 days' " .T₂ = 21 " " .D₀ = No disinfection.D₁ = Disinfection with 1 mg Usputun powder pr g seed.D₂ = Disinfection with 2 mg Usputun powder pr g seed.

dried for two days and was sown on May 14th. They were both sown at Ultuna on loamy soil. Very bad drought occurred during the weeks after sowing, and sprouting was very uneven. Shooting started on some plots about July 20th, and the number of ears on September 21st is given in table 2. Kernels developed in a few ears which were harvested on October 7th, giving a total weight of 31 g. The plot which gave the greatest number of ears is shown in fig. 2.

Experiments in 1937.

Experiments in 1936 had shown that a chilling period of 21 days gave the greatest number of ears per plot. Therefore in the 1937 experiments with chilling periods of 20, 30 and

a) The field experiment.

Plan of experiment.

Plot	Symbols ¹			Plot	Symbols ¹			Plot	Symbols ¹		
	W.	D.	T.		W.	D.	T.		W.	D.	T.
a/1	0	0	1	b/1	1	0	1	c/1	2	0	1
a/2	1	1	1	b/2	2	1	1	c/2	0	1	1
a/3	2	2	1	b/3	0	2	1	c/3	1	2	1
a/4	1	0	2	b/4	2	0	2	c/4	0	0	2
a/5	2	1	2	b/5	0	1	2	c/5	1	1	2
a/6	0	2	2	b/6	1	2	2	c/6	2	2	2
a/7	2	0	3	b/7	0	0	3	c/7	1	0	3
a/8	0	1	3	b/8	1	1	3	c/8	2	1	3
a/9	1	2	3	b/9	2	2	3	c/9	0	2	3

¹ W₀ = Without water.

W₁ = 50 % " .

W₂ = 60 % " .

D₀ = No disinfection.

D₁ = Disinfection with 1 mg Uspulun powder
pr g seed.

D₂ = Disinfection with 2 mg Uspulun powder
pr g seed.

T₁ = 20 days' chilling at 1—2° C.

T₂ = 30 " " " 1—2° C.

T₃ = 40 " " " 1—2° C.

40 days were used. The seed was chilled in a frigidaire at 1—2° C and received water (50 % and 60 % respectively) at 9 a.m., 12 a.m. and 3 p.m., each time 1/3 of the total. Between the water treatments the seed was stirred twice. It was placed in the frigidaire at 5 p.m. the same day. Two experiments were sown, one in the field, and one in Mitscherlich pots in the cage at the Institute.

On May 14th the experiment was sown according to the block method, the area of each plot being 10 sq.m (2 m × 5 m). The weather was very dry both before and after sowing, and sprouting was poor. Although only a few ears appeared during the summer it was clear that vernalization for 30 or 40 days gave the greatest number of ears.

b) The pot experiment.

The plan of the experiment is given in table 3. The surface area of the pots was 0.03 sq.m. Seed from the yield of the 1936 vernalization experiments was employed. Sowing

Table 3.

Results of pot experiment in 1937.

Treatments	Symbols ¹			Number of ears on Aug. 25th	Height of plants ² on Aug. 9th cm	Yield in grammes ²		Total weight of kernels g	Number of kernels	Mean weight of kernels g
	W.	D.	T.			Straw	Seed			
5	1	0	1	4	95	5.10	0.33	0.99	69	0.014
6	1	1	1	7	103	10.43	0.43	1.29	80	0.016
7	1	0	2	—	—	—	—	—	—	—
8	1	1	2	10	115	9.80	2.40	7.20	292	0.025

¹ W₀ = Without water.

D₀ = No disinfection.

W₁ = 50 % " .

D₁ = Disinfection with 1 mg Uspulun powder pr g seed.

T₀ = Without chilling.

T₁ = 20 days' chilling at 1—2° C.

T₂ = 30 " " " 1—2° C.

² Average for three replications.

took place on May 19th and sprouting on May 27th. No plants appeared after treatment 7, as the kernels had been killed by fungi during vernalization. Shooting started on July 12th after 10 days vernalization and on July 21st after 20 days vernalization. Ripening took place on September 9th. Table 3 gives the results of the experiment. Treatments 1—4 did not induce any shooting and are therefore omitted in the table.

Experiments in 1938.

In the experiments in 1935 to 1937 the best results were obtained from vernalization with the longest periods of chilling, and therefore long chilling periods were planned for the experiments in 1938. The water was given in the same way as in 1937 but the seed was left at 20° C, until the following morning and then placed in a frigidaire at 1—2° C. In the previous years the vernalization had been done in vessels covered with glass plates and the ventilation had been rather limited. In 1938 the vessels were not covered, but water was added at short intervals in such a way as to keep the moisture constant throughout the whole vernalization period. One experiment was sown in the field and two in pots in the cage.

Field experiment.

Plan of experiment.

1. No treatment.
2. Disinfection, 1 mg Uspulun powder pr g seed.
3. Vernalization 40 days.
4. Disinfection and vernalization 40 days.
5. Vernalization 50 days.
6. Disinfection and vernalization 50 days.
7. Vernalization 60 days.
8. Disinfection and vernalization 60 days.

All vernalized seed received 50 % water. Each treatment was replicated four times, the area of the plots measuring 9 sq. m. (3 × 3 m). Since experience in previous years had shown that

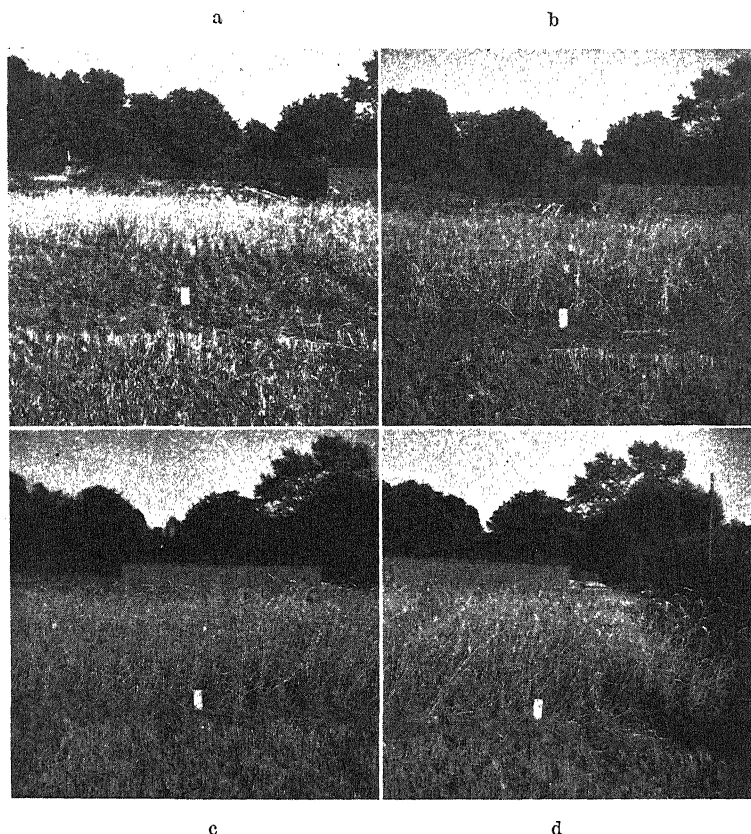


Fig. 3. Vernalization experiment with Stålråg.

- a) Disinfected and not vernalized seed.
- b) " " 40 days vernalized seed.
- c) " " 50 " " "
- d) " " 60 " " "

Photo 8/9 1938.

early spring sowing might induce better sprouting, the 1938 experiment was sown on April 29th. Sprouting was good and the stand was uniformly dense. Shooting commenced in the first weeks of July. The results of the experiment are

given in table 4. Date of ripening for treatments 7 and 8 was the 10th of Sept.; for 5 and 6 the 12th of Sept. and for 3 and 4 the 14th of Sept. The average measurement for plant height in treatments 3—8 was 110 cms and in treatments 1—2 about 70 cms.

Table 4.

Results of field experiment in 1938.

Treatment	Number of ears on Sept. 9th		Straw weight		Weight of seed		Grain offal %	Weight of 1 000 kernels g
	Total	Fertile	kg/ha	Rel.	kg/ha	Rel.		
1	63	4	—	—	—	—	—	—
2	36	2	—	—	—	—	—	—
3	484	350	622	100	156	100	14.3	21.7
4	621	486	711	114	167	107	13.3	21.2
5	806	651	833	134	244	156	13.6	21.5
6	1 090	833	1 189	191	289	185	15.4	20.8
7	979	791	1 000	161	278	178	16.0	22.3
8	883	703	856	138	211	135	15.8	21.1

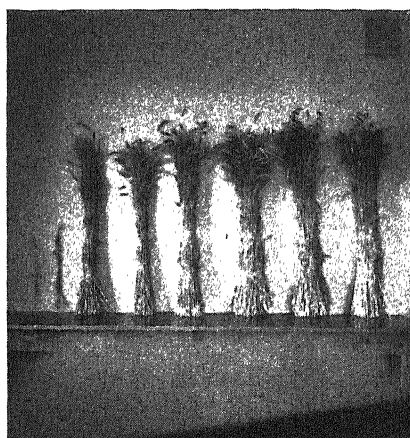


Fig. 4. The yield from the a-plots of the field experiment in 1938. To the left the yield from the two plots sown with untreated seed, then follows the yield from plots sown with seed vernalized in 40, 50 and 60 days. The sheaves are arranged according to the plan of experiment on page 78.

Pot experiments.

a) **Experiment No. 1.** For this experiment the seed harvested from the vernalization experiments in 1937 was used.

Plan of experiment.

1. No treatment.
2. Disinfection, 1 mg Uspulun powder pr g seed.
3. Vernalization 40 days.
4. Disinfection and vernalization 40 days.
5. Vernalization 50 days.
6. Disinfection and vernalization 50 days.
7. Vernalization 60 days.
8. Disinfection and vernalization 60 days.

The water given was 50 % of the seed weight. Each treatment was replicated. The experiment was sown on May 5th; sprouting was good and the density uniform. Shooting began in the middle of July (treatments 5—8). The results of the experiment are given in table 5. Ripening occurred on the 14th of Sept. after treatments 7 and 8 and on the 15th of Sept. after 5 and 6.

Table 5.

Results of pot experiment No. 1.

Treatments	Number of ears on Sept. 14th		Height of plants Sept. 2nd cm	Straw weight		Weight of seed		Seed weight g
	Total	Fertile		g/pot	Rel.	g/pot	Rel.	
1	10	3	55	3.15	84	0.05	15	0.010
2	4	1	45	2.05	54	0.01	3	0.008
3	6	2	70	3.77	100	0.34	100	0.020
4	15	7	70	5.63	149	0.42	124	0.014
5	8	7	110	7.63	202	1.08	303	0.021
6	11	6	100	6.66	177	1.14	335	0.020
7	7	6	115	9.43	250	2.33	685	0.028
8	8	6	115	9.73	258	1.78	524	0.023

b) Experiment No. 2.

Plan of experiment.

- | | |
|--------------------------|-----------------------------|
| 1. Seed not treated. | 4. Seed vernalized 60 days. |
| 2. " vernalized 40 days. | 5. " " 70 " . |
| 3. " " 50 " . | |

The seed was not disinfected. In all treatments 50 % water was given. Each treatment was replicated twice. The experiment was sown on May 5th, and sprouting was uniform. Shooting started during the first half of July. The results of the experiment are given in table 7. After treatment 5) ripening occurred on Sept. 13th, and after treatment 4) on Sept. 19th.

Table 6.

Results of pot experiment No. 2.

Treatments	Number of ears on Sept. 14th		Height of plants Sept. 2nd cm	Straw weight		Weight of seed		Seed weight g
	Total	Fertile		g/pots	Rel.	g/pots	Rel.	
1	4	1	58	1.82	39	0.08	35	0.016
2	9	3	78	4.67	100	0.28	100	0.017
3	9	3	90	5.30	114	0.14	61	0.012
4	12	6	102	9.95	213	1.68	730	0.028
5	14	12	115	17.64	378	5.33	2 317	0.027

Discussion.*The effect of disinfection.*

The experiments in 1935 had clearly shown the importance of using good seed for vernalization. The only explanation of the big differences in density between the various treatments seems to be that vernalization had a deleterious effect on the seed. As a consequence investigations on germination and sprouting of differently vernalized seed were carried out in the winter 1935—36. GESCHELE (1932) is of the opinion that in using disinfectants there is a danger of stimulating growth, too intensive for the seed. In the investigations in

1935—36 good results were obtained from a disinfection with Uspulun powder, particularly in vernalizations with 50 % water. The best results were obtained with 1 mg Uspulun powder per gramme seed, the largest quantity used in these investigations. The germination percentage after vernalization in 20 days with 50 % water of seed disinfected with 1 mg per gramme was only 2.1 % lower than the germination percentage of seed not vernalized. But in the case of seed not disinfected and vernalized under the same conditions the germination percentage was 29.1 % lower. Similar results were obtained concerning the sprouting power. — From seed not vernalized 87 plants (out of 100) sprouted and 12 were infected by *Fusarium* or *Penicillium*. Seed vernalized 20 days with 50 % water gave 89 and 22 plants respectively when disinfected with 1 mg Uspulun powder per gramme seed, but 70 and 89 resp. when not disinfected.

Between the plots sown with disinfected and not disinfected seed in 1936 no difference was observed. In 1937, however, although the conditions were very severe, there was an obvious difference between disinfected and not disinfected seed. On plots sown with seed not disinfected and vernalized with a high percentage of water (50—60 %) the stand was rather light but on plots sown with disinfected seed vernalized under the same conditions the stand was dense. Similar results were obtained in the pot experiments of the same year and in all experiments in 1938. Disinfection with Uspulun powder obviously is advantageous for the sprouting as well as for the density, the question is, however, if such a disinfection is in any way unfavourable to the effect of vernalization.

The effect of vernalization.

In 1935 only a few ears appeared and no kernels developed. Ears appeared only from vernalized seed. In 1936 the experiments showed quite obviously that more ears appeared from vernalized seed than from seed not vernalized. Differences could also be observed between different methods of vernalization. 50 % water and a chilling period of 21 days

gave the best result. The drying of the seed after vernalization did not appear to affect the results. In 1937 the field experiment was badly affected by drought immediately after the sprouting, and only a few ears developed. There was, however, a tendency to increasing number of ears after vernalization with long chilling periods. In the pot experiments the same year, for which the yield from the experiments in 1936 was used, ears were obtained from seed disinfected with 1 mg Uspulun powder per gramme seed and vernalized for 30 days with 50 % water. An average of 2.40 grammes of seed was obtained from three pots. The kernels were slightly smaller than usual. Although the experiments up to 1937 had not given very conclusive results they indicated that vernalization had an effect on Stålråg. In addition, they showed that the disinfection of seed, necessary for a satisfactory density, did not have a deleterious influence in any way and, further, that a relatively high water percentage should be employed and that the number of ears increased with the length of the chilling period. Therefore, in the 1938 experiment 50 % water, disinfection with Uspulun powder and chilling for 40, 50, 60 and 70 days were used. Previously the sowing had taken place in the middle of May, but according to the plan in 1938, sowing took place at the end of April. In the field experiments that year the yield of seed varied from 156 to 289 kg/ha. These figures are very low as compared with those from an ordinary crop but they provide sufficient evidence of improvement in the vernalization method that year. The weight of a thousand kernels, 20.8 to 22.3 g, is low as compared to the normal weight, 30 to 35 g. The yield and the thousand kernels weight seem to indicate that many details of the method employed should be investigated closer. Judging from the pot experiments still longer chilling periods might be employed with good results. Better conditions for vernalization, in these experiments only a frigidaire or a dark cellar have been available, seem to be necessary to obtain better results.

The results seem to indicate that the Swedish rye variety Stålråg requires long chilling periods; considerably longer than the period of three weeks or one month which is usually regarded as normal. This coincides principally with the Russian opinion that the optimum length of the vernalization period depends upon the climate of the district of origin of the varieties. It seems probable that the Swedish varieties require a chilling period of two months or more. Disinfection of the seed is necessary. Vernalized seed should be sown only when the environmental conditions are suitable.

Sammanfattning.

Åren 1935—1938 utfördes vid institutionen för växtodlingslära vid Lantbrukshögskolan försök med jarovisering av höstråg (Stålråg). Första året, 1935, användes obetat utsäde, som jaroviserades med 40 resp. 50 % vatten vid en temperatur av ca 3° C under 25 dagar. Uppkomsten av det jaroviserade utsädet blev dålig och det bildades endast ett fåtal ax, i vilka ingen kärnsättning skedde. Under vintern 1935—36 gjordes en undersökning av betning av jaroviserat utsäde med Uspulun torrbetningsmedel, varvid framkom god effekt av betningen. Sålunda hade grobarheten efter 20 dagars jarovisering med 50 % vatten hos utsäde betat med 1 mg Uspulun pr gram utsäde endast nedgått med 2.1 % i förhållande till det ej jaroviserade utsädet grobarhet men med 29.1 hos obetat utsäde. Skjutkraftsundersökningarna visade samma tendens. Det ej jaroviserade utsädet gav 87 uppkomna och 12 *Fusarium*- eller *Penicillium*-smittade plantor, medan det som jaroviserats 20 dagar med 50 % vatten gav 89 resp. 22, då det betats med 1 mg/g Uspulun men 70 resp. 89 då det ej betats.

I fältförsök sommaren 1936 användes obetat och på olika sätt betat utsäde. Betningen invercade detta år icke på uppkomsten och beståndet. Axskjutningen blev något bättre än föregående år, varvid den i försöket använda längsta kylningsperioden, 21 dagar, gav bästa resultatet. Mogen skörd erhöles fastän i mycket liten mängd.

År 1937 utlades ett försök på fält och ett i Mitscherlich-kärl i vegetationsgården. Båda försöken gävo mycket goda utslag till förmån för betningen, beroende på starka påfrestningar efter sådden. I fältförsöket blevo dessa så starka, att beståndet blev dåligt och ett mycket litet antal ax framkom, i vilka inga kärnor bildades. I kärkförsöket erhöles däremot såväl ax som kärnor, och det visade sig även detta år att den längsta kylningsperioden, som nu var 30 dagar, gav högsta resultatet, 2.40 g kärnor pr kärl (markyta = 0.03 m^2) i medeltal från tre upprepningar. Kärnstorleken var något mindre än normal, vikten var 0.025 g.

I de försök, som utlades år 1938, ett fältförsök och två kärkförsök, användes för jaroviseringen långa kylningsperioder 40, 50, 60 och 70 dagar. I två av försöken jämfördes obetat och Uspulunbetat utsäde, varvid erhöles samma resultat som förut. Den långa kylningstiden visade sig vara fördelaktig, och i fältförsöket erhöles skördar, varierande från 156—289 kg/ha. Den lägsta skörden erhöles efter utsäde, som jaroviserats 40 dagar och de högsta efter det, som jaroviserats 50 och 60 dagar. I kärkförsöken erhöles överensstämmande resultat, de högsta skördarna vid jarovisering 60 och 70 dagar. Tusenkornvikten varierade mellan 20.8 och 22.3 g.

Av försöken, som hittills varit rent orienterande, har framgått nödvändigheten av betning av utsädet. Denna betning kan utföras med Uspulun torrbetningsmedel. Vidare synas långa kylningstider (2 månader eller mera) vara nödvändiga för jarovisering av svenska höstsädessorter.

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Über die Einwirkung der Kalidüngung auf die Festlegung der Phosphorsäure im Boden.

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Einleitung.

Die Festlegung der Phosphorsäure im Boden hat in den letzten Jahren in allen Kulturländern wiederholt die Aufmerksamkeit der Bodenwissenschaftler erregt, und kaum wurde wohl ein anderes Gebiet der Bodenkunde in letzter Zeit so häufig in der Literatur behandelt (GEHRING 1931). Die Löslichkeit der Phosphorsäure bzw. die Festlegung derselben ist vor allem von dem pH-Wert des Bodens, dem Verhältnis zwischen aktiven Azidoiden und Basoiden desselben sowie dem Kalkgehalt abhängig (GAARDER 1930 und 1935, HIBBARD 1935, MATTSON & KARLSSON 1938, u. a.).

Dass die Phosphorsäurefestlegung auch in schwedischen Böden oft sehr stark ist, geht aus den Arbeiten von FRANCK (1935 & 1937) und TORSTENSSON & ERIKSSON (1938 a; b) hervor, obwohl die Festlegung auf verschiedenen Böden ganz verschieden gross ist (ERIKSSON 1938).

Auch das Kali wird besonders in kolloidreichen Böden meistens stark festgelegt, so dass man mit einer Auswaschung von Kaliumsalzen in der Regel nicht zu rechnen braucht (ECKSTEIN 1935). Während die Phosphorsäure, wenn man die Ausdrücke von MATTSON verwendet, in unseren schwedischen Böden meistens »extra-mizellar» hauptsächlich an Ca gebunden

sein dürfte, wird das Kali in weitem Ausmasse durch Austausch-adsorption von den Bodenkolloiden festgelegt. Diese letztere Erscheinung soll nach VAGELER durch eine Hyperbel wiedergegeben werden können, wobei er jedoch sicher voraussetzt, dass der Boden in vertikaler Richtung gleichförmig ist.

Die Frage, wie Kali- und Phosphorsäuredüngemittel in bezug auf Zugänglichkeit auf einander wirken, ist auch vereinzelt in der Literatur Gegenstand eines gewissen Interesses gewesen, obwohl man über den gegenseitigen Einfluss dieser beiden Düngemittel nirgends klaren Bescheid erhält. Manchmal wird es jedoch möglich sein, in der Literatur aus den Untersuchungen anderer Probleme hier und da gewisse Daten zu dieser Frage zu finden. Da es auch wertvoll sein dürfte, die vorliegenden Untersuchungen allseitiger zu beleuchten, wird in dieser Arbeit auch die betreffende Literatur berücksichtigt.

In ihrem Handbuch der künstlichen Düngemittel geben v. NOSTITZ und WEIGERT (1928) ältere Daten an, nach denen die Löslichkeit der Phosphorsäure in Thomasmehl durch Vermischen desselben mit Kainit wesentlich erhöht werden könne. Diese Behauptungen sind aber von FISHER (1932) auf Grund eingehender Untersuchungen widerlegt worden. FISHER schliesst seine Arbeit mit folgenden Worten. »Es wird sich also empfehlen, die in Frage stehende Erscheinung in der neueren Literatur nicht mehr aufzuführen, da eine Steigerung der Thomasmehlphosphorsäure durch Vermischen mit Kainit nicht erreicht wird und zu Irreführungen Anlass geben kann.»

Schon in den Jahren 1904 und 1907 publizierten CAMERON und Mitarbeiter (CAMERON & HURST 1904; CAMERON & BELL 1907) Arbeiten über die Wirkung von Wasser und wässrigen Salzlösungen auf Bodenphosphate. Besonders interessant ist in diesen Arbeiten, dass u. a. die Löslichkeit der Eisen- und Aluminiumphosphate in Neutralsalzlösungen verschiedener Stärke untersucht worden ist. In Tabelle 1 sind einige der Daten dieser Autoren zusammengestellt, die die Wirkung steigender Mengen KCl auf die PO_4 -Löslichkeit aus Eisenphosphat zeigen.

Tabelle 1.

Eisenphosphat in Lösungen von KCl. (CAMERON & BELL aus Tabelle XXXIV.)

Lösung per g Eisen- phosphat	‰ KCl in der Lösung	Lösliche PO ₄	Lösung per g Eisen- phosphat	‰ KCl in der Lösung	Lösliche PO ₄
20 cm ³	0	0,3500	40 cm ³	0	0,2175
»	0,111	0,2256	»	—	—
»	0,272	0,2011	»	—	—
»	0,444	0,1689	»	—	—
»	5,0	0,1127	»	5	0,0930
»	25	0,0905	»	25	0,0769
»	75	0,0820	»	75	0,0569
»	150	0,0871	»	150	0,0523
»	300	0,0871	»	300	0,0428

Auch andere Analysendaten gehen in derselben Richtung. Mit K₂SO₄ anstatt KCl haben diese Autoren keine ähnlichen Resultate für Eisenphosphat bekommen. Bei Aluminiumphosphat dagegen zeigt nicht nur KCl, sondern auch K₂SO₄ und NaNO₃ die oben illustrierte Tendenz, dass die Löslichkeit mit der Konzentration der Neutralsalzlösung abnimmt. Einige dieser Daten sind in Tab. 2 wiedergegeben.

Da diese Tabelle aus Daten verschiedener Originaltabellen besteht, in denen das Ausgangsmaterial heterogen ist, sind nur Zahlen innerhalb jeder Kolumne unter sich vergleichbar. Einige weitere Resultate der zitierten Autoren sind in der Tab. 3 wiedergegeben. Hier ist auch die Wirkung des Kaliumchlorids auf die Phosphatlöslichkeit des Ca₃(PO₄)₂ ersichtlich.

Auch in dieser Tabelle sind die PO₄-Werte nur innerhalb der Kolumnen vergleichbar. Die Verfasser sagen schon damals in ihrer Zusammenfassung der Resultate: »The presence of potassium chloride in the solution decreases the amount of phosphoric acid obtained from iron, aluminium or calcium

Tabelle 3.

Die Wirkung des Kaliumchlorids auf die PO_4 -Löslichkeit einiger Phosphate.¹ 1 g. Phosphat pro 20 cm³ Lösung.

(Aus Tab. VII; XV und XXV in CAMERON & HURST 1904).

Eisenphosphat		Aluminiumphosphat		Kalziumphosphat	
g KCl pro Liter	lösliche PO_4 pro Liter	g KCl pro Liter	lösliche PO_4 pro Liter	g KCl pro Liter	lösliche PO_4 pro Liter
0	0,8500	0	0,241	5	0,3278
5	0,1127	150	0,201	25	0,3227
25	0,0905	200	0,189	75	0,3193
150	0,0871	250	0,186	300	0,2356

phosphates.» Zu irgendwelchen für die Praxis nützlichen Resultaten kamen die betreffenden Wissenschaftler in dieser Frage nicht.

Nach den Untersuchungen von DREISPRING und HEINRICH (1931) über die Erhöhung der Wurzellöslichkeit des Bodenkalis durch Düngerphosphorsäure sind auch andere Forscher dazu angeregt worden, das Umgekehrte experimentell zu untersuchen, d. h. nachzuprüfen, ob die Kalidüngung eventuell eine günstige Wirkung auf die Phosphatzugänglichkeit der Böden ausübte (ENGELS 1932 & 1933). DREISPRING und HEINRICH'S Untersuchungen wurden hauptsächlich nach der Neubauerschen Keimpflanzenmethode ausgeführt. Sie haben gezeigt, dass eine Steigerung der Wurzellöslichkeit des Bodenkalis durch die Düngerphosphorsäure »unverkennbar« ist, und sie glauben auf Grund anderer Untersuchungen (FROHWEIN-BOMLITZ 1927), dass die Phosphorsäure durch eine Kalidüngung (KNO_3) für die Keimpflanzen mobil gemacht wird.¹

¹ Ein näheres Studium der zitierten Arbeit zeigt aber, dass dies nur bei Kaliumnitrat der Fall war und dass bei Verwendung von Kaliumchlorid sogar eine kleine Depression der aufgenommen P_2O_5 bemerkbar ist.

ENGELS fand in Keimpflanzenuntersuchungen nach der Neubauerschen Methode, dass ein Zusatz von Kainit die P_2O_5 -Aufnahme meistens deutlich erhöhte. Er will aber daraus keine für die Praxis sichere Schlüsse ziehen.

CHAPMAN (1936) schliesst nach Resultaten von Gefässversuchen, dass physiologisch saure Stickstoffdüngemittel die Phosphatzugänglichkeit in kalkreichen Böden erhöhen, und v. WRANGEL (1926) fand in Laboratorienuntersuchungen, dass in Neutralsalzlösungen die P_2O_5 -Löslichkeit von tertiärem Ca-Phosphat grösser war als in destilliertem Wasser, und zwar, dass diese Löslichkeit bei Ammoniumsalzen stärker war als bei Alkaliesalzen und dass weiter die Sulphate im allgemeinen eine stärker lösende Wirkung ausübten als die entsprechenden Chloride. Ähnlicher Meinung war schon GREAVES (1910), als er schrieb: »Sodiumsulphate, calciumsulphate, ammoniumsulphate, ammoniumchloride, ammoniumnitrate, and magnesiumnitrate render phosphates more soluble» und weiter, dass »the effect of magnesiumsulphate, sodiumchloride, magnesiumchloride and potassiumchloride is small or varies with the different phosphates.»

FREY (1933) hat die Phosphatlöslichkeit in einem alten Kalidüngungsversuch geprüft und fand dabei in den kaligedüngten Parzellen eine grössere Quantität lösliches P und dies war ganz besonders der Fall, wenn KCl verwendet worden war.

Die alten Untersuchungen von CAMERON u. a. (1904 & 1907) werden jedoch von den hier erwähnten Resultaten nicht überzeugend widerlegt. Sie werden im Gegenteil von mehreren neueren Arbeiten und auch von unseren eigenen Untersuchungen bestätigt. So fanden z. B. McGEORGE und BREAZEALE (1931) wenn sie den Boden mit KCl-Lösungen behandelten, die in Tabelle 4 wiedergegebenen Daten.

BLANCK und Mitarbeiter (1931) haben für Kalidüngung in Gefässversuchen mit schwach gepuffertem Odersand eine kleinere Depression des Ernteertrages erhalten, die auf Phosphatfestlegung zurückgeht.

Es gibt auch Keimpflanzen-Untersuchungen, die im Gegen-

Tabelle 4.

Die Wirkung von Kaliumchlorid auf die Löslichkeit von Bodenphosphaten.

					‰ PO_4 in Lösung
Wässriger Bodenextrakt					1,2
»	»	KCl	1 g pro Liter		0,3
»	»	»	2	»	0,2
»	»	»	10	»	Spur
»	»	»	20	»	Spur
»	»	»	50	»	Spur

satz zu den oben erwähnten von ENGELS eine deutliche Depression der aufgenommenen Phosphorsäure bei K_2O -Zusatz zeigen (KROSS 1927, Vergl. auch LOHSE 1930). Die folgende Tabelle (Tab. 5) ist nach den Daten von KROSS aufgestellt.

Tabelle 5.

Wirkung der Kalidüngung auf die Aufnahme von Phosphorsäure. Keimpflanzenmethode.

Phosphatdüngung	Kalidüngung	Boden aus Dahlhem (Schwach humoser lehmiger Sand)		Boden aus Markee (milder Lehm Boden)	
		P_2O_5 Aufnahme	Zu- oder Abnahme bei K_2O -Düngung	P_2O_5 Aufnahme	Zu- oder Abnahme bei K_2O -Düngung
Nur Bodenphosphorsäure	0	6,4	—	9,8	—
	1 K	5,9	—0,5	7,7	—1,6
	2 K	6,5	+0,1	6,4	—2,9
Bodenphosphorsäure + 20 mg P_2O_5	0	18,4	—	14,5	—
	1 K	15,9	—2,5	12,7	—1,8
	2 K	15,7	—2,7	11,0	—3,5
Bodenphosphorsäure + 40 mg P_2O_5	0	24,5	—	14,6	—
	1 K	20,5	—4,0	13,3	—1,3

Diese Arbeit zeigte ausserdem, dass eine mit der Kali-düngung gleichzeitige Stickstoffgabe die P_2O_5 -Aufnahme begünstigt. In mehreren Fällen ergibt jedoch die Steigerung der K_2O -Düngung auch bei gleichzeitiger N-Gabe eine Depression der aufgenommenen Phosphorsäure.

LOHSE & RUHNKE (1933) haben die Phosphatlöslichkeit von Mono- und Dikalziumphosphaten in der Ackerkrume eines Bodens aus Charing Cross, Kent County, unter Zusatz mehrerer verschiedenen Düngersalze untersucht. Die Phosphatuntersuchungen sind nach der $KHSO_4$ -Methode gemacht. Durchgehend wird das $Ca(H_2PO_4)_2$ aq. in der Bodensubstanz stärker festgelegt als $CaHPO_4$ 2 aq. Die Neutralsalze, u. a. KCl und das Ammoniumsulphat, verursachen alle eine Abnahme der wiedergefundenen Phosphorsäure, während basische Düngemittel z. B. Kalkstickstoff beim erstgenannten eine kräftige Zunahme bewirken.

Dass verschiedene Forscher abweichende Resultate für die Wirkung der Salze auf die Löslichkeit der Phosphate, resp. auf die Festlegung der Phosphorsäure im Boden bekommen haben, wird aus den obigen Daten deutlich hervorgehen. BUEHRER (1932) macht eine Zusammenstellung der qualitativen Resultate einiger Forscher, die hier wieder gegeben wird. Die P-Löslichkeit aus Kalziumphosphaten in einigen Salzlösungen wird als mehr oder weniger löslich in Vergleich zu derjenigen in destilliertem Wasser angegeben.

Diese Tabelle könnte an Hand der oben erwähnten Daten leicht ergänzt werden. Der zitierte Autor schreibt, nachdem er konstatiert hat, dass die betreffenden Angaben in der Literatur »somewhat conflicting» sind: »Some investigators find that a certain salt increases phosphate solubility while others find the opposite to be true.»

Worauf beruht nun diese wenig ermunternde Unstimmigkeit der bisher vorliegenden Daten? Natürlich zu gewissem Grade auf der angewandten Untersuchungsmethodik, aber zweifelsohne liegt darin nicht die ganze Wahrheit. Beim Arbeiten mit Böden spielen nach der Meinung des Verfassers die Ver-

Tabelle 6.

Die Löslichkeit von Kalziumphosphaten in Neutralsalzlösungen (nach BUEHRER).

S a l z	A u t o r			
	CAMERON & BELL	GREAVES	V. WRANGEL	McGEORGE & BREAZEALE
KCl	weniger	weniger	mehr	weniger
NaCl	mehr	weniger	mehr	weniger
CaCl ₂	weniger	viel weniger	—	weniger
NaNO ₃	mehr	etwas mehr	mehr	—
Ca(NO ₃) ₂	weniger	weniger	—	—
K ₂ SO ₄	—	mehr	mehr	weniger
(NH ₄) ₂ SO ₄	—	mehr	mehr	—
CaSO ₄	mehr	weniger	—	weniger
CaCO ₃	weniger	—	—	weniger

schiedenheiten der Böden eine grosse Rolle. RAUTERBERG (1931 & 1932) betont, dass beim Beurteilen der Phosphatfestlegung eines Bodens vor allem die physikalische Beschaffenheit desselben berücksichtigt werden muss, wobei es besonders darauf ankommt, dass die kolloidalen Mineralbestandteile quantitativ bestimmt werden. Die organischen Bestandteile scheinen dabei nicht besonders berücksichtigt worden zu sein.

Natürlich sind die rein physikalischen Eigenschaften des Bodens, d. h. in diesem Falle seine Textur und Mikrostruktur, von grosser Bedeutung, aber die grösste Wirkung auf die Phosphatfestlegung werden doch die physikalisch-chemischen Eigenschaften der verschiedenen Böden haben.

Bei einer vorbereitenden Untersuchung (ERIKSSON 1938) hat der Verfasser feststellen können, dass auf einem sauren phosphatarmen Lehm Boden die Phosphatfestlegung aus Superphosphat bei Vermischung desselben mit 40-prozentigem Kalisalz gesteigert wurde. In gleicher Richtung gehen die Durchlässigkeitsversuche von MIDGLEY (1931), die ergaben, dass das

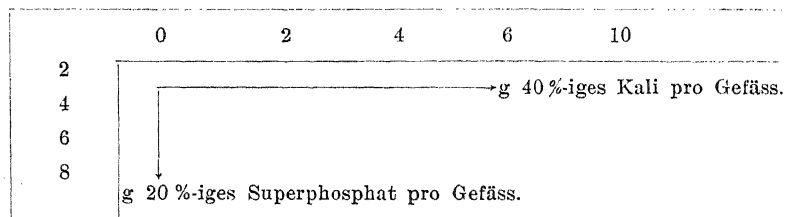
Sickerwasser weniger Phosphorsäure enthielt, wenn der durchsickerte Boden mit einer Mischung von Superphosphat und K_2SO_4 gedüngt war als bei Düngung mit blossem Superphosphat. ERIKSSON hat diese Erscheinung mit den Befunden von MATTSON (1928) über Aktivierung von Fe und Al durch Neutralsalze in Zusammenhang gebracht. Die positive Wirkung eines Neutralsalzzusatzes bei der Auslösung dieser beiden Metalle aus dem Boden ist auch in deutlicher Weise von PAVER und MARSHALL (1934) gezeigt worden. Eigene Untersuchungen bestätigen diese Tatsache.

Vorbereitende Untersuchungen.

Die Böden, mit denen wir hier anfangs arbeiteten, sind saure Lehm Böden, die etwas gyttjahaltig sind und die relativ viel aktives Eisen enthalten. Der Phosphatbedarf sowie die Phosphatfestlegung dieser Böden ist stark und seit Jahren arbeiteten wir am hiesigen Institut für allgemeine Ackerbaulehre an dem Problem, diese Schwierigkeiten zu bemeistern (TORSTENSSON & ERIKSSON 1938). Um so interessanter war es, als wir 1938 in einem durch die obenerwähnte präliminäre Untersuchung veranlassten Gefässversuch, konstatieren konnten, dass der Phosphatmangel der Pflanzen auf diesen Böden durch Kalidüngung gesteigert wurde.

Dieser Gefässversuch (Nr. 27, 1938) wurde in Mitscherlichgefässen nach einem Plan durchgeführt, der steigende Mengen Superphosphat resp. 40 %-iges Kali vorsah.

Plan des Gefässversuches Nr. 27, 1938.



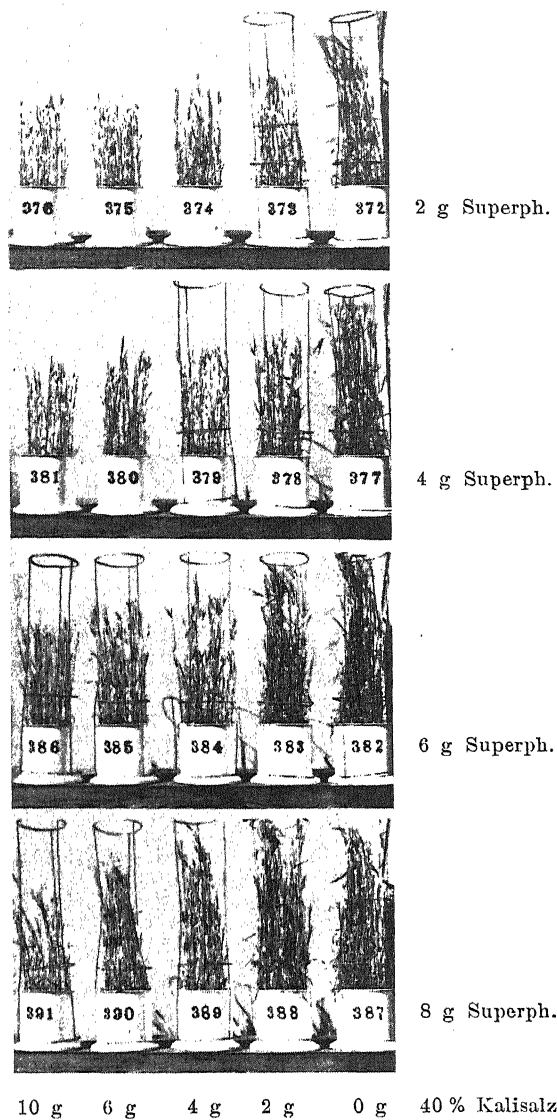


Abb. 1. Versuch 27/1938 mit steigenden Mengen Phosphorsäure und Kalisalz.

Tabelle 7.

Kornertrag des Versuches 27/1938. g. Trockensubstanz pro Gefäss.

Düngung pro Gefäss	g 40 %-iges Kali pro Gefäss					
Superphosphat g	0	2	4	6	10	Durchschn.
2	7,27	6,10	3,88	2,17	2,19	4,31
4	12,19	6,92	4,84	4,74	2,26	6,19
6	18,96	13,24	7,58	5,92	3,71	9,88
8	14,74	18,16	10,54	10,04	2,48	11,19
Durchschnitt	13,29	11,11	6,70	5,72	2,66	7,89

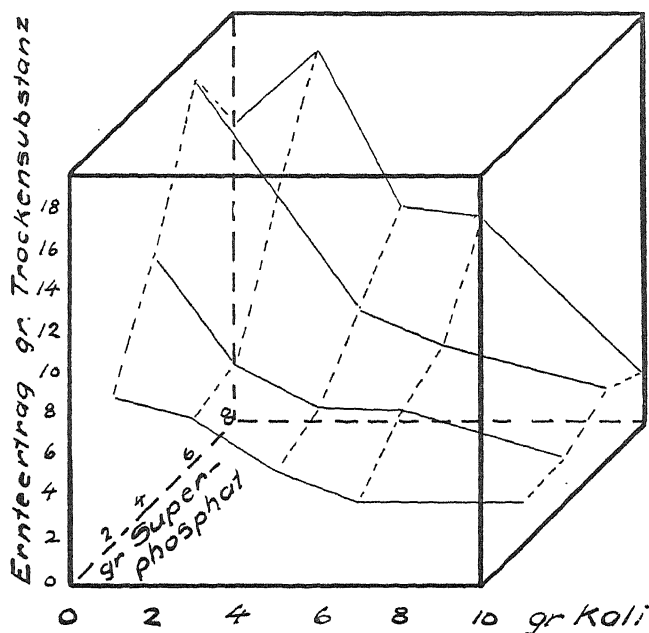


Diagramm 1.

Stereometrische Darstellung des Ernteertrages von Versuch 27/1938.

Ausser der Versuchsdüngung wurde noch 3,5 g Ammoniumnitrat pro Gefäss gegeben. Der Boden, der, nach der N. J. F.¹-Nomenklatur, als ein humushaltiger bis humusreicher, schwerer Lehm Boden mit kleiner Beimischung von Gytjtja bezeichnet werden kann, wurde benutzt, ohne dass Zusatzmittel zu seiner physikalischen Verbesserung gegeben worden wären. Der pH-Wert des Bodens war am Anfang des Versuches 5,0. Als Versuchspflanze diente Gerste (Svalöfs Goldgerste), die sich in früheren Versuchen als ein guter Indikator des Phosphatzustandes dieser Böden erwiesen hatte.

Schon in einem sehr frühen Stadium der Pflanzenentwicklung war deutlich zu sehen, dass bei gleicher Phosphatdüngung die kaligedüngten Gefässe schwächer entwickelt waren als die nicht kalidüngten, und zwar um so schwächer, je mehr Kali sie bekommen hatten.

Diese von der Kalidüngung verursachte Depression war während der ganzen Vegetationsperiode an der vegetativen Entwicklung der Pflanze klar zu sehen und spiegelte sich auch im Kornertrage deutlich ab. Abb. 1 zeigt die verschiedenen Gefässe gleich nach dem Schossen. Der Ernteertrag wird in der Tabelle 7 und in Diagramm 1 wiedergegeben.

Der mittlere Fehler der Differenz zwischen den Kolumnen der Tab. 7 ist $\pm 1,79$ und zwischen den Reihen $\pm 1,60$. Die Ausrechnung ist nach der in TORSTENSSON & ERIKSSON (1938 a) beschriebenen Methode ausgeführt worden. Dass eine Steigerung der Phosphatdünger bei diesem phosphatbedürftigen Boden eine Erhöhung des Ernteertrages verursachte, war nur zu erwarten. Aber dass die Kalidüngung eine so kräftige und durchgehende Depression verursachte, ist um so bemerkenswerter und sehr interessant. Die Differenz $6,59 \pm 1,79$ zwischen 0 und 4 g 40 %-iges Kalisalz pro Gefäss ist ganz sicher, und noch grösser wird die Differenz zwischen »Ohne Kali« und den grösseren Kaligaben. Das Diagramm zeigt auch anschaulich, wie die Steigerung der Kalidüngung durchgehends eine sukzessive Depression des Ernteertrages verursacht. Dass es

¹ In Übersetzung: Verein Nordischer Landwirtschaftlicher Forscher.

sich nicht um eine Kalivergiftung handelt, dürfte ja erstens aus den relativ kleinen absoluten Mengen der Kalidüngung hervorgehen und zweitens wird eine solche Vermutung durch die Versuche des Jahres 1939 widerlegt (siehe Seite 106).

Im Jahre 1939 wurde die Nachwirkung der Düngung von 1938 geprüft. Die Festlegung der kleineren Mengen Phosphorsäure war während der zwei Jahren beinahe absolut geworden und der Kornertrag war, wie Tabelle 8 zeigt, bei den mässig P-gedüngten ganz unbedeutend. Bei 8 g Superphosphat findet man aber Gefässe, die einen bedeutend besseren, obwohl noch nicht normalen Ertrag zeigen.

Die einzige Reihe in diesem Versuch, die eine so grosse Ernte gegeben hat, dass man irgendwelche Schlüsse daraus ziehen kann, ist die letzte mit 8 g Superphosphat. Es geht aus ihr hervor, dass eine kleine Kaligabe eine Erhöhung des Ernteertrages bewirkt, die höheren Gaben aber Abnahmen verursachen. Dies ist kein Zufall und dieses Problem wird in Zusammenhang mit den Versuchen von 1939 eingehender diskutiert.

Tabelle 8.

Nachwirkung des Versuches 27/1938 in Jahre 1939.
Kornertrag an Gerste in g pro Gefäss.

g Superphosphat pro Gefäss	g 40 %-iges Kali pro Gefäss				
	0	2	4	6	10
2	0,53	0,56	0,80	0,95	0,69
4	0,76	0,56	0,83	0,95	1,38
6	2,84	2,94	1,77	1,81	2,74
8	3,50	5,26	2,08	2,16	1,21

Zu den Daten des Versuches 27 der beiden Jahre sei nur noch hinzugefügt, dass die Erträge von Stroh etwa in der gleichen Richtung gehen wie die hier angegebenen Kornerträge.

Gefäßversuche im Jahre 1939.

Im Sommer 1939 wurden die Untersuchungen erweitert. Grössere Versuche wurden angesetzt und die Resultate des Jahres 1938 wurden auch an einigen anderen Böden nachgeprüft. Der vollständigste Versuch wurde auf demselben Boden durchgeführt wie Versuch 27/1938. Dieser Versuch 10/1939 wurde nicht wie der vorher besprochene in Mitscherlichgefässen, sondern in Tonröhren mit einer lichten Weite von 15,5 cm und einer inneren Länge von 61 cm durchgeführt. Eine nähere Beschreibung dieser selbstfabrizierten Gefässe ist in der schon zitierten Arbeit von TORSTENSSON und ERIKSSON (1938 a) gegeben. Als Versuchspflanze diente auch diesmal Svalöfs »Goldgerste«. Der Versuchsplan war wie folgt:

Versuch 10/1939.

Gefäss Nr.	Versuchsdüngung pro Gefäss.				
1	2 g	Superphosphat (gewöhnliches)			bis zu 10 cm Tiefe einge- mischt.
2	4 g	»	»		
3	6 g	»	»		
4	8 g	»	»		
5	10 g	»	»		
6	2 g	»	»	+ 4 g 40 % Kalisalz	
7	4 g	»	»	»	
8	6 g	»	»	»	
9	8 g	»	»	»	
10	10 g	»	»	»	
11	2 g	»	»		in einer Schicht bei 5 cm.
12	4 g	»	»		
13	6 g	»	»		
14	8 g	»	»		
15	10 g	»	»		
16	2 g	»	»	+ 4 g Kalisalz	
17	4 g	»	»	»	
18	6 g	»	»	»	
19	8 g	»	»	»	
20	10 g	»	»	»	

Gefäss Nr.

Versuchsdüngung pro Gefäss.

21	2 g Superphosphat (gekörnertes)				bis zu 10 cm Tiefe eingemischt.
22	4 g	»	»		
23	6 g	»	»		
24	8 g	»	»		
25	10 g	»	»		
26	2 g	»	»	+ 4 g Kalisalz	
27	4 g	»	»	»	
28	6 g	»	»	»	
29	8 g	»	»	»	
30	10 g	»	»	»	
31	2 g	»	»		in einer Schicht bei 5 cm.
32	4 g	»	»		
33	6 g	»	»		
34	8 g	»	»		
35	10 g	»	»		
36	2 g	»	»	+ 4 g Kalisalz	
37	4 g	»	»	»	
38	6 g	»	»	»	
39	8 g	»	»	»	
40	10 g	»	»	»	
41	4 g Kalisalz + 14 Tage später 2 g gew. Superph.				bis zu 10 cm eingemischt.
42	»	»	4 g	»	»
43	»	»	6 g	»	»
44	»	»	8 g	»	»
45	»	»	10 g	»	»
46	»	»	2 g gekörnt. Superph.	»	»
47	»	»	4 g	»	»
48	»	»	6 g	»	»
49	»	»	8 g	»	»
50	»	»	10 g	»	»
51	2 g Kalisalz + 4 g gew. Superphosphat				»
52	4 g	»	»		»
53	6 g	»	»		»
54	8 g	»	»		»
55	10 g	»	»		»

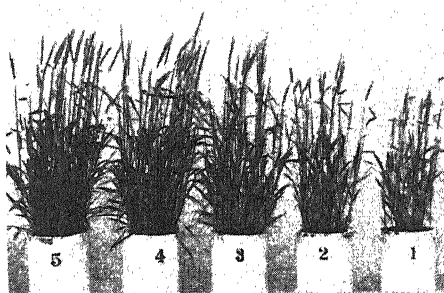
Gefäss Nr.	Versuchsdüngung pro Gefäss.		bis zu 10 cm eingemischt.
56	2 g Kalisalz + 4 g gekörnt. Superphosphat		»
57	4 g	»	»
58	6 g	»	»
59	8 g	»	»
60	10 g	»	»
61	2 g	» + 8 g gew. Superphosphat	»
62	4 g	»	»
63	6 g	»	»
64	8 g	»	»
65	10 g	»	»
66	2 g	» + 8 g gekörnt. Superphosphat	»
67	4 g	»	»
68	6 g	»	»
69	8 g	»	»
70	10 g	»	»

In allen Fällen, in denen Superphosphat und Kalisalz gleichzeitig gegeben wurden, wurden diese beiden Düngemittel vorher mit einander vermischt. Ein paar Serien sind auch mit aufgenommen, in welchen das Kali 14 Tage vor der Phosphorsäure gegeben wurde. In diesem Falle wurde der Boden schon vor der Kalidüngung mit Wasser bis zu 60 % der Wasserkapazität versetzt. In den Serien, wo der Dünger in einer Schicht von 5 cm Tiefe gelegt wurde, ist darauf geachtet worden, dass bei der Saat diese Schicht nicht zerstört wurde.

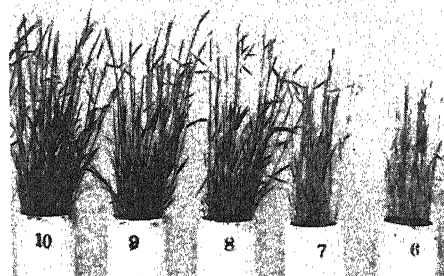
Die Körnergrösse des von uns in diesem Versuch benutzten gekörnten Superphosphats bewegte sich zwischen 1—4 mm.

Der Übersichtlichkeit halber wird bei den folgenden Erörterungen Versuch 10/1939 in drei Teile zerlegt, die 10 a, 10 b und 10 c bezeichnet werden. Zu 10 a werden die Serien mit steigenden Mengen gewöhnlichen Superphosphats, zu 10 b die mit steigenden Mengen gekörnten Superphosphats und zu 10 c diejenigen mit steigenden Mengen 40 % Kalisalze gerechnet. Abb. 2 zeigt die Entwicklung der Serie mit gewöhnlichem

Nur Superph.
bis zu 10 cm
eingemischt.



Superph. plus
4 g Kali zu
10 cm ein-
gemischt



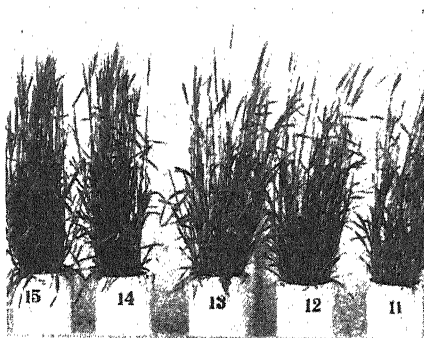
10 g 8 g 6 g 4 g 2 g Superphosphat

Abb. 2. Einbringung von gewöhnlichem Superphosphat ohne und mit Vermischung mit 40 %-igem Kalisalz.

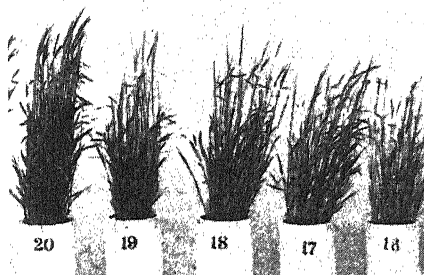
Superphosphate, etwa 14 Tage vor der Reife bis zu 10 cm eingemischt, verglichen mit der Serie, die dazu noch 4 g 40 % Kalisalz bekommen hat. Die kräftigere Entwicklung der nur phosphatgedüngten Momente ist deutlich wahrzunehmen. Noch deutlicher tritt die deprimierende Wirkung des Kalis in Abb. 3 hervor, die den entsprechenden Vergleich zwischen den Serien zeigt, in denen der Dünger in einer Schicht bei 5 cm Tiefe plaziert wurde.

Der Kornertrag in g Trockensubstanz pro Gefäß wird in der Tabelle 9 wiedergegeben. Die Ziffern in der Klammer geben den entsprechenden Strohertrag an.

Superphosphat
in einer Schicht
bei 5 cm. Tiefe.



Superphosphat
plus 4 g Kali
in einer Schicht
bei 5 cm. Tiefe.



10 g 8 g 6 g 4 g 2 g Superphosphat

Abb. 3. Platzierung von Superphosphat ohne und mit Vermischung von 40 %-igem Kalisalz bei 5 cm Tiefe.

Die Daten der Kornerträge sind auch in Diagramm 2 graphisch wiedergegeben, nachdem sie nach einer Parabel von der Form

$$y = a + bx + cx^2$$

ausgeglichen worden sind.¹

Kurve 1 und 2 können verglichen werden. Die durchgehends

¹ In der Wirklichkeit folgen die Ernteerträge wahrscheinlich nicht einer ähnlichen Parabelkurve, aber da es hier nicht die Absicht ist, den absoluten Verlauf der Kurven anzugeben, sondern sie nur so darzustellen, dass sie unter sich vergleichbar sind, ist es zweckmässig, wenn man sich die Ertragskurven innerhalb der untersuchten Grenzen als Parabeln vorstellt.

Tabelle 9.

Ernteertrag des Versuches 10 a/1939. g Trockensubstanz pro Gefäß von Korn und in der Klammer g lufttr. Stroh.
Gewöhnliches Superphosphat.

Art der Einbringung und ev. K-Düngung	P-Düngung g pro Gefäß					
	2	4	6	8	10	Durch- schn.
Superphosphat bis zu 10 cm eingemischt	15,03 (24)	16,16 (17)	29,82 (28)	42,43 (38)	39,34 (34)	28,6
Superphosphat und 4 g Kali bis zu 10 cm eingemischt .	6,14 (13)	8,91 (16)	22,02 (25)	25,61 (28)	30,45 (30)	18,6
Superphosphat in einer Schicht bei 5 cm Tiefe	31,68 (32)	41,47 (43)	48,23 (45)	50,06 (47)	61,60 (58)	46,6
Superph. und 4 g Kali in einer Schicht bei 5 cm Tiefe . .	18,55 (21)	31,91 (36)	34,11 (35)	33,48 (41)	46,33 (50)	32,9
4 g Kali und 14 Tage später Superph. bis zu 10 cm . .	3,83 (7)	7,90 (11)	17,26 (21)	27,67 (30)	29,83 (31)	17,3

niedrigere Ernte bei Kurve 2 beruht auf der negativen Wirkung des Kalidüngers. Ebenso klar liegt die entsprechende Wirkung bei einem Vergleich zwischen den Kurven 3 und 4 am Tage. Dass diese beiden höher liegen als die beiden erstgenannten, steht ganz in Übereinstimmung mit unseren früheren Untersuchungen (TORSTENSSON & ERIKSSON 1938) und ist eine gute Bestätigung der damaligen Resultate, die ergaben, dass die technisch richtige Applizierung des Düngers für die Wirkung desselben von grösster Bedeutung ist.

Dass es sich in den kaligedüngten Serien um Phosphatmangel handelt und nicht um Kalivergiftung — das letztere wäre auch bei so kleinen Kaligaben auf diesem sehr stark gepufferten Boden unmöglich — ist ganz klar, da Kurve 4 ebensoviel Kalisalz repräsentiert wie Kurve 2, aber trotzdem höher verläuft als Kurve 1. Wenn man, wie der Verfasser immer

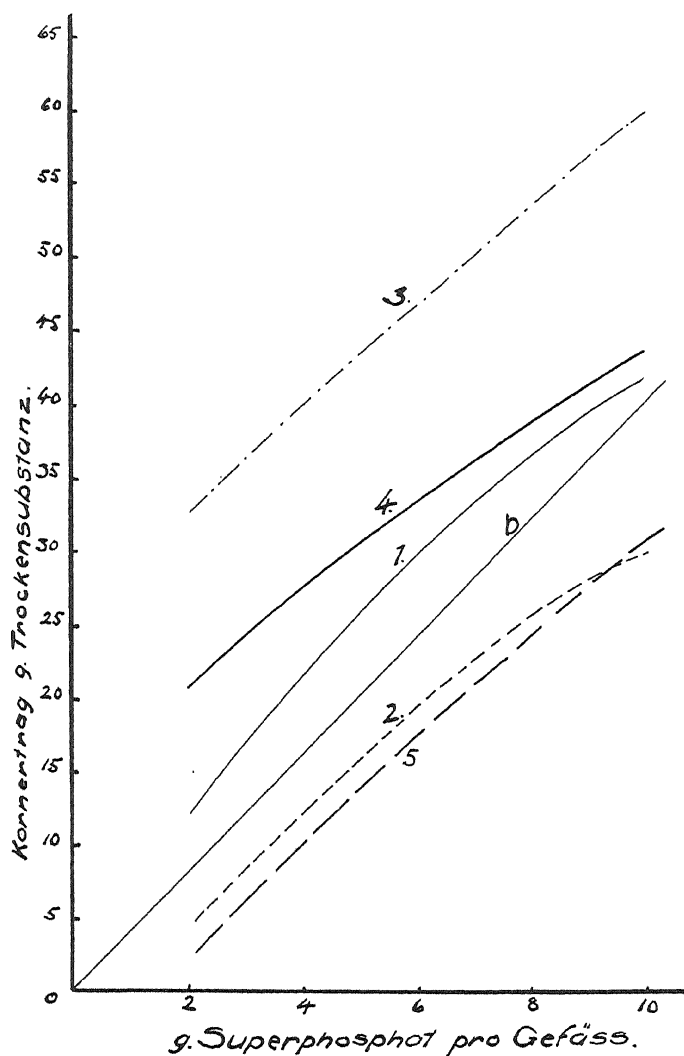


Diagramm 2. Versuch 10 a/1939.

1. Superph. bis zu 10 cm eingemischt.
2. Superph. + Kali bis zu 10 cm eingemischt.
3. Superph. in einer Schicht bei 5 cm Tiefe.
4. Superph. + Kali in einer Schicht bei 5 cm Tiefe.
5. Zuerst Kali, 14 Tage später Superph. bis zu 10 cm eingemischt.

noch meint, annehmen kann, dass das Eisen und Aluminium des Bodens von dem KCl des Kalidüngers aktiviert wird, können diese Erscheinungen erklärt werden. Auch der niedrige Verlauf der Kurve 5 könnte dann dadurch erklärt werden, dass das Kalisalz in diesem Falle längere Zeit für seine Wirkung auf die Eisenverbindungen u. a. des Bodens gehabt hat, so dass die Phosphorsäure sofort nach der Einmischung im Boden als Eisenphosphat u. dgl. anstatt z. B. als Kalziumtriphosphat festgelegt wird.

Die Differenzen zwischen den Durchschnittszahlen der Reihen in Tabelle 9 hat einen mittleren Fehler von $\pm 3,26$ d. h. bei Einmischung bis zu 10 cm Tiefe hat die Kaligabe den Kornertrag um $10,0 \pm 3,26$ g und bei Plazierung bei 5 cm um $13,7 \pm 3,26$ g gesenkt. Die Differenzen sind also sicher. Beim Vergleich zwischen »Superphosphat plus Kali, bis zu 10 cm eingemischt«, und »Superphosphat allein in einer Schicht bei 5 cm Tiefe« — die Differenz ist $28,0 \pm 3,26$ — verdient es beachtet zu werden, dass es sich um dieselben Mengen gewöhnlichen Superphosphats handelt.

Derselbe Versuch ist in Versuch 10 b/1939 mit gekörntem Superphosphat wiederholt. Die Phosphatmengen pro Gefäss waren in diesem letzteren Versuche in bezug auf wasser- und zitratlösliches P_2O_5 äquivalent zu denjenigen des vorher beschriebenen. Die Ernteerträge sind in Tabelle 10 wiedergegeben. Die Erträge an Stroh, sind, um die Tabellen handlicher zu machen, in diesem und den meisten der folgenden Versuchen nicht mit aufgenommen. Es muss jedoch gesagt werden, dass dieses Auslassen der Strohdaten nicht auf die Auffassung der Resultate einwirkt. Im Gegenteil bestätigen diese die Schlussfolgerungen, die aus den Korndaten gezogen worden sind.

Die Daten des Versuches 10 b/1939 sind, nachdem sie in der früher beschriebenen Weise ausgeglichen worden sind, in Diagramm 3 dargestellt.

Auch hier begegnet dasselbe Bild. Der Kalizusatz bei Einmischung der Dünger bis zu 10 cm hat den Kornertrag durch-

Tabelle 10.

Ernteertrag des Versuches 10 b/1939. g Trockensubstanz pro Gefäss. Gekörntes Superphosphat.

Art der Einbringung und ev. K-Düngung	P-Düngung g					
	2	4	6	8	10	Durch- schn.
bis zu 10 cm eingemischt . .	22,52	25,56	42,85	54,69	54,41	39,9
mit 4 g Kali bis zu 10 cm eingemischt	10,86	28,17	37,83	44,23	50,07	34,2
in einer Schicht bei 5 cm Tiefe	34,88	53,75	64,11	40,51	58,18	50,3
mit 4 g Kali in einer Schicht bei 5 cm Tiefe	15,56	33,78	36,88	37,95	48,28	34,5
4 g Kali und 14 Tage später Superphosphat bis zu 10 cm	5,08	20,75	23,71	26,19	30,87	21,3

schnittlich um $5,7 \pm 4,23$ herabgesetzt und bei Platzierung der Dünger in einer Schicht bei 5 cm Tiefe wurde der Ertrag durchschnittlich um $15,8 \pm 4,23$ gedrückt. Auch in diesem Versuche läuft die Kurve für die Reihe mit der 14 Tage vor der Phosphatdüngung gegebenen Kalidüngung am niedrigsten. Die Erklärung des Verhältnisses zwischen den Kurven ist natürlich dieselbe wie in Versuch 10 a.

Um einen Vergleich zwischen den Resultaten der Versuche 10 a und 10 b zu erleichtern, ist die Bisektrix des Origowinkels auf beiden Diagrammen eingelegt worden. Ein derartiger Vergleich zeigt, dass die Kurven des letzten Versuches höher liegen als die des ersten. Dies beruht, wie schon in der vorher zitierten Arbeit (TORSTENSSON & ERIKSSON 1938 a) gesagt wurde, auf der Überlegenheit des gekörnten Superphosphats auf derartigen Böden.

In Versuch 10 c/1939 wurde die Phosphatgabe innerhalb der Serien konstant gehalten, während die Kalidüngung gesteigert wurde. Das Ergebnis dieses Versuches ist in Tabelle 11 und Diagramm 4 vorgeführt.

Die sukzessive Abnahme der Ernte bei steigendem Kalizu-

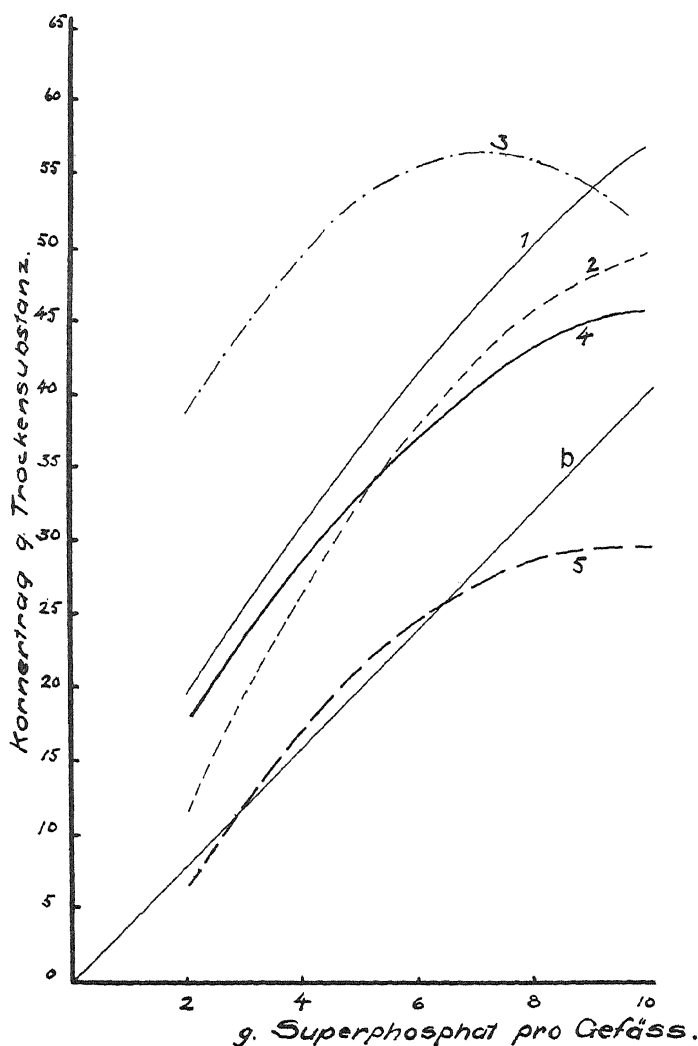


Diagramm 3. Versuch 10 b/1939.

1. Superph. bis zu 10 cm eingemischt
2. Superph. + Kali bis zu 10 cm eingemischt.
3. Superph. in einer Schicht bei 5 cm Tiefe.
4. Superph. + Kali in einer Schicht bei 5 cm Tiefe.
5. Zuerst Kali, 14 Tage später Superph. bis zu 10 cm eingemischt.

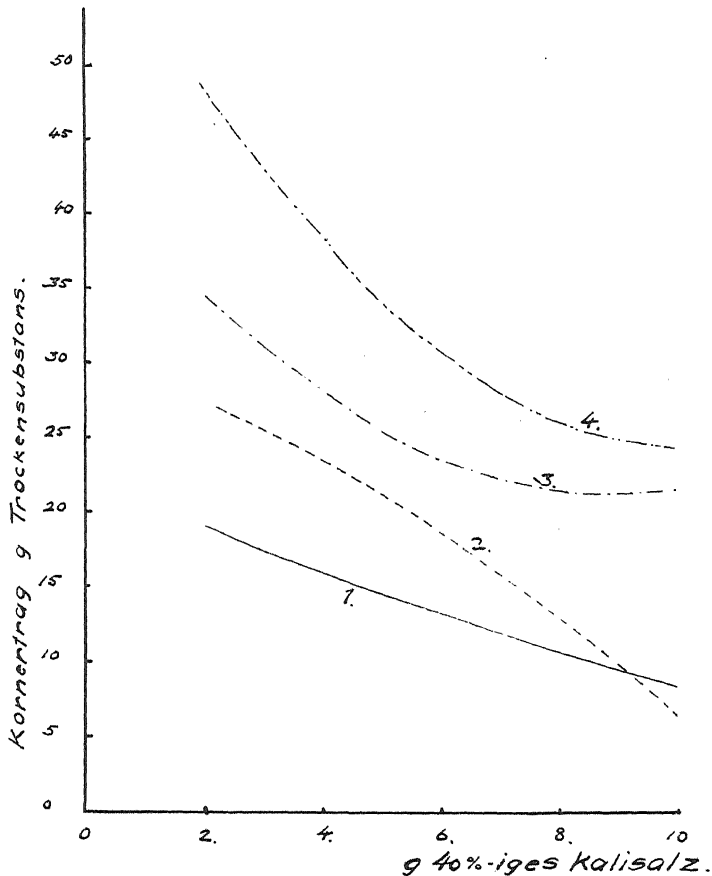


Diagramm 4. Versuch 10 c/1939.

1. 4 g gew. Superphosphat bis zu 10 cm eingemischt.
2. 4 g gekörnt. Superphosphat bis zu 10 cm eingemischt.
3. 8 g gew. Superphosphat bis zu 10 cm eingemischt.
4. 8 g gekörnt. Superphosphat bis zu 10 cm eingemischt.

satz ist in allen vier Reihen durchgehend. Eine direkte Kalivergiftung kommt auch bei der höchsten Kalimenge kaum in Frage. Es kann in dieser Beziehung daran erinnert werden, dass der Boden ein stark gepufferter schwerer Lehm Boden ist. Ausserdem liegt ja in Tab. 11 der niedrigste Wert der letzten

Tabelle 11.

Ernteertrag des Versuches 10 c/1939. g Trockensubstanz
pro Gefäss.

Phosphatdüngung	K a l i d ü n g u n g					Durch- schn.
	2	4	6	8	10	
4 g gewöhnl. Superphosphat bis zu 10 cm	20,22	14,50	10,07	16,20	6,20	13,4
4 g gekörn. Superphosphat bis zu 10 cm	26,31	25,87	20,54	8,66	8,55	18,0
8 g gewöhnl. Superphosphat bis zu 10 cm	33,66	31,66	18,11	25,46	20,85	26,0
8 g gekörnt. Superphosphat bis zu 10 cm	49,81	35,97	32,58	26,66	23,69	33,7
Durchschnitt	32,5	27,0	20,3	19,3	14,8	

Reihe höher als der höchste Wert der ersten Reihe. In dieser Tabelle ist der mittlere Fehler der Differenz zwischen den Durchschnittszahlen zweier Spalten $\pm 3,24$ und der zweier Reihen $\pm 2,89$.

Auch hier ist die Überlegenheit des gekörnten Düngers deutlich wahrzunehmen. Eine Anzahl Körnerproben von Versuch 10 a, b und c sind auf dem Totalgehalt des aufgenommenen P_2O_5 und K_2O untersucht worden. Die Analysendaten, in denen kleinere Schwankungen vorkommen, zeigen keine regelmässige Veränderungen im prozentuellen Inhalt von P_2O_5 und K_2O . Die aufgenommenen Mengen dieser beiden Nährstoffe scheinen also, soweit man aus den Kornanalysen schliessen darf, proportional zu dem Ernteertrag zu sein.

Um das Problem etwas weiter zu beleuchten, wurden im Sommer 1939 ähnliche Versuche wie 27/1938 auf einigen Böden in gewöhnlichen Mitscherlichgefässen durchgeführt. Die Nummer dieser Gefässversuche waren 4, 5, 11 und 12. Der eine Boden, Versuch 4/1939, war ein Humusboden — Glühverlust 50,3 % und pH-Wert 5,1 — der in einem früheren Feldversuch

einen grossen Ausschlag für Phosphorsäure, aber einen negativen für Kali ergeben hatte. Der Boden von Versuch 5/1939 war ein humusarmer, moglemischer mittelschwerer Lehm Boden, also ein reiner Mineralboden, von gutem Kalkzustand und einem pH-Wert von 7,7. Der Boden, der in den Versuchen 11/1939 und 12/1939 benutzt wurde, ähnelte dem Boden des oben beschriebenen Versuches 10, d. h. er war ein mittelschwerer Lehm Boden mit ein wenig Gytja. Der pH-Wert war 5,1. Auf diesem letztgenannten Boden wurden zwei Versuche durchgeführt, der eine mit gewöhnlichem pulverförmigen Superphosphat und der andere mit äquivalenten Mengen gekörntem Superphosphats. Der Plan, nach dem die vier Versuche angesetzt wurden, war:

Plan der Versuche Nr. 4, 5, 11 und 12/1939.

	0	2	4	6	10
0	→ g 40 %-iges Kalisalz pro Gefäss				
2					
4					
6					
8					
15	↓ g 20 %-iges Superphosphat pro Gefäss				

Als Versuchspflanze diente auch in diesen Versuchen Goldgerste. Ausser der Versuchsdüngung wurde für eine etwa optimale Stickstoffgabe gesorgt, indem 3,5 g Ammoniumnitrat pro Gefäss gegeben wurde. Sowohl die Versuchsdüngung wie die Stickstoffdüngung wurde in diesen Versuchen gleichmässig im ganzen Gefäss eingemischt.

Während des grössten Teils der Vegetationsperiode konnte man in den Versuchen 4, 11 und 12 ein Optimum der Entwicklung für kleinere Kaligaben in jeder Reihe der verschiedenen Phosphatgaben feststellen und dieses Optimum schien sogar mit grösserer Phosphatdüngung in der Richtung höherer Kaligaben verschoben zu sein.

Die Ernteerträge des Versuches 4/1939 werden in Tabelle 12 und Diagramm 5 wiedergegeben. Der Phosphatbedarf ist somit auf diesem Boden sehr gross, aber auch kleinere Kalimengen scheinen den Ertrag zu begünstigen. Und zwar: je mehr Phosphorsäure der Boden bekommen hat, desto grösser ist der Ausschlag für Kali und desto weiter nach rechts liegt das Maximum der Kurven, d. h. desto mehr Kalisalz kann zugeführt werden, ohne dass eine negative Wirkung der Kalidüngung auftritt. Der mittlere Fehler ist in Tabelle 12 für die Differenzen zwischen zwei Reihen $\pm 1,44$ und zwischen zwei Spalten $\pm 1,31$.

Tabelle 12.

Kornertrag des Versuches 4/1939. g Trockensubstanz pro Gefäss.

Phosphat- düngung g	K a l i d ü n g u n g g					
	0	2	4	6	10	Durchschnitt
0	6,73	7,15	7,07	6,81	5,77	6,7
2	26,57	31,42	30,88	29,37	27,69	29,1
4	35,41	36,21	38,99	40,29	36,63	37,5
6	33,81	40,36	43,26	44,83	42,29	40,9
8	33,97	39,49	44,92	41,26	41,16	40,2
15	37,42	43,08	40,48	44,64	46,58	42,4
Durchschnitt	29,0	33,0	34,2	34,5	33,4	32,8

Versuch 5/1939 zeigt überhaupt keine sicheren Differenzen mit Ausnahme zwischen »Phosphatgedüngt« und »Nicht Phosphatgedüngt«. Die Ergebnisse dieses Versuches sind in der Tabelle 13 zu finden.

Die Reihe mit 0 g Superphosphat zeigt eine unerwartet grosse Steigerung für Kalidüngung. Sonst scheint das Kali nicht wesentlich auf das Resultat eingewirkt zu haben. Eine negative Kaliwirkung war ja nach den hier lancierten Theorien

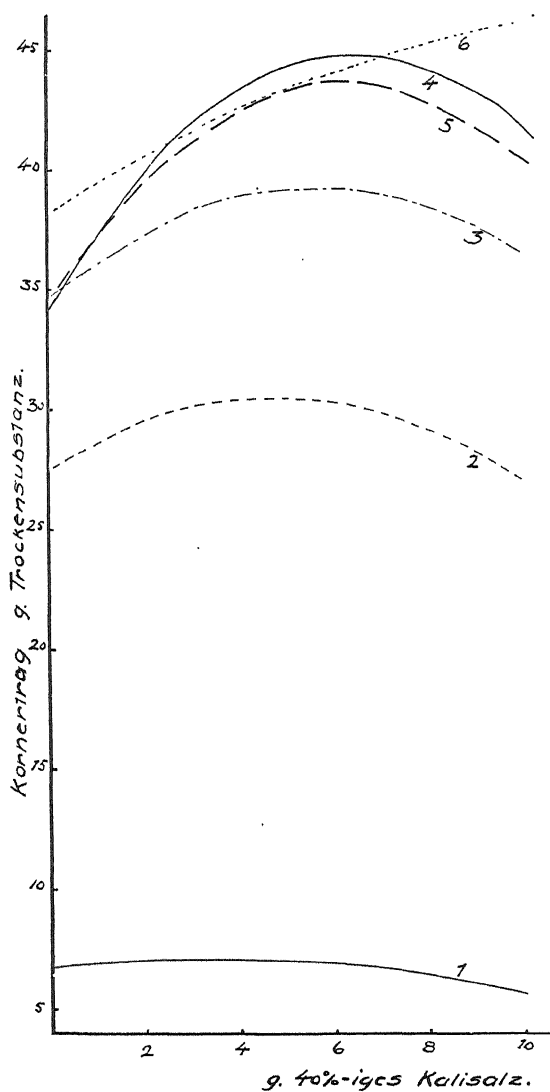


Diagramm 5. Versuch 4/1939.

- | | |
|------------------------|-----------------------|
| 1. Ohne Superphosphat. | 4. 6 g Superphosphat. |
| 2. 2 g „ | 5. 8 g „ |
| 3. 4 g „ | 6. 15 g „ |

Tabelle 13.

Kornertrag des Versuches 5/1939. g Trockensubstanz
pro Gefäss.

Phosphat- düngung g	K a l i d ü n g u n g g					
	0	2	4	6	10	Durch- schnitt
0	5,60	24,62	34,43	33,02	32,41	26,0
2	40,55	41,92	45,00	41,12	40,22	41,8
4	41,36	43,73	45,50	43,67	45,24	43,9
6	42,16	46,47	36,69	42,03	42,78	42,0
8	45,41	44,82	45,49	41,02	42,77	43,9
15	45,74	38,32	38,01	46,60	45,12	42,8
Durchschnitt	36,8	40,0	40,9	41,2	41,4	40,1

auf diesem reinen Mineralboden, der beinahe Ca-gesättigt ist, nicht zu erwarten.

Die Versuche 11 und 12 werden am besten beide zusammen besprochen, da es sich um denselben Boden handelt. Die Resultate dieser beiden Versuche sind in den Tabellen 14 und 15 sowie in den Diagrammen 6 resp. 7 wiedergegeben. Da es von Interesse sein kann, auch die Stroherträge zu studieren, sind diese für Versuch 11 in der Tabelle mit aufgenommen. Die Stroherträge des Versuches 12 gehen in derselben Richtung.

Die Resultate der beiden Versuche sind einander sehr ähnlich, nur dass das gekörnte Superphosphat durchgehends etwas höhere Ernten ergeben hat. Auch die Kurven der beiden Diagramme sind einander sehr ähnlich. Wenn wir zuerst die kleinste Phosphatgabe der beiden Versuche, 2 g pro Gefäss, betrachten, finden wir, dass die Kalidüngung eine Depression der Ernte verursacht. Die Erklärung wird hier dieselbe sein wie in Versuch 10. Da entsteht die Frage: Wie kommt es, dass die Kurven für höhere Phosphatgaben auf demselben Boden eine deutliche Ertragssteigerung für kleine Kaligaben zeigen, während bei kleineren Phosphatgaben die Kalidüngung

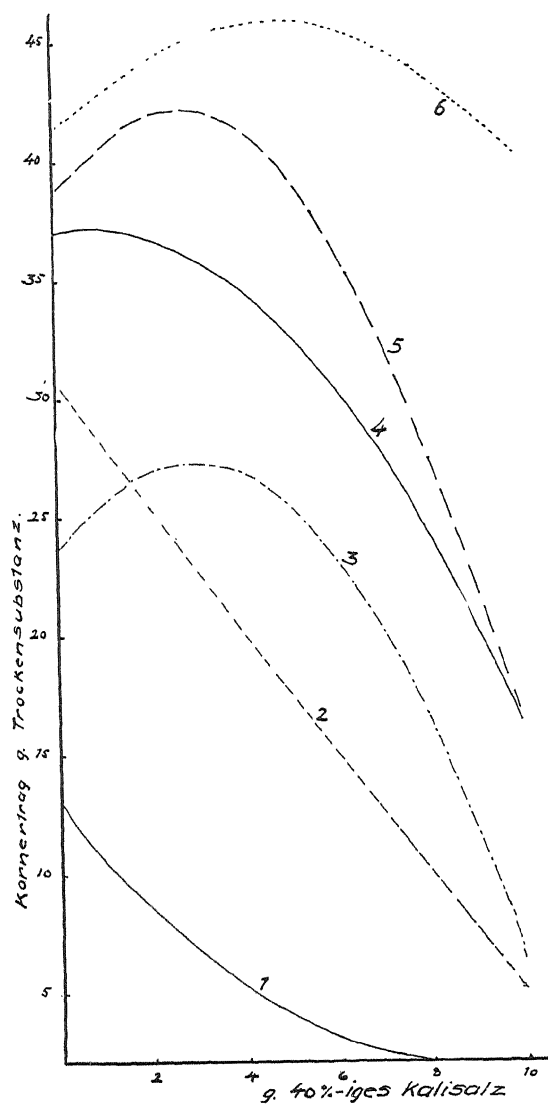


Diagramm 6. Versuch 11/1939.

- | | |
|----------------------------|----------------------------|
| 1. Ohne Superphosphat. | 4. 6 g Superphosphat. |
| 2. 2 g " | 5. 8 g " |
| 3. 4 g " | 6. 15 g " |

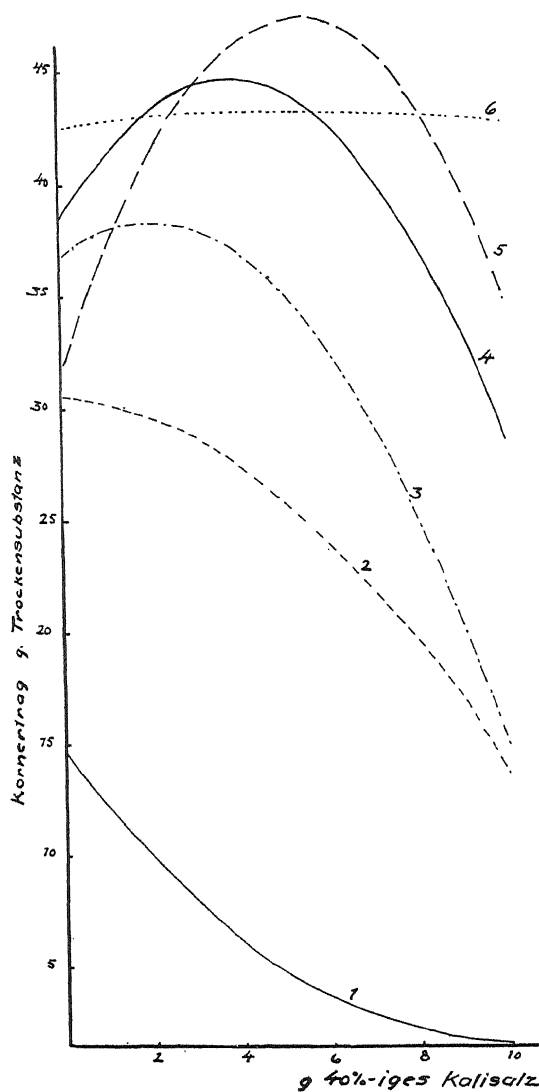


Diagramm 7. Versuch 12/1939.

- | | |
|-----------------------------|-----------------------------|
| 1. Ohne Superphosphat. | 4. 6 g Superphosphat. |
| 2. 2 g " | 5. 8 g " |
| 3. 4 g " | 6. 15 g " |

Tabelle 14.

Ernteertrag des Versuches 11/1939. g Trockensubstanz pro Gefäss von Korn und in der Klammer g. lufttr. Stroh.

Phosphat- düngung g	K a l i d ü n g u n g g					
	0	2	4	6	10	Durch- schnitt
0	12,74 (17)	9,48 (13)	4,64 (8)	3,27 (6)	2,22 (7)	6,5
2	30,28 (33)	26,61 (31)	21,19 (25)	13,41 (18)	5,64 (14)	19,4
4	19,06 (26)	33,52 (39)	31,46 (34)	14,71 (21)	8,62 (15)	21,5
6	35,07 (40)	39,90 (46)	36,20 (42)	27,34 (33)	17,36 (23)	31,2
8	38,91 (43)	40,57 (48)	42,79 (50)	36,49 (42)	15,79 (23)	34,9
15	40,08 (42)	45,48 (53)	48,27 (59)	42,34 (48)	41,17 (44)	43,5
Durchschnitt	29,4	32,6	30,8	22,9	15,1	26,2

durchgehends eine Depression herbeiführt. Dies wird sehr einfach dadurch erklärt, dass der Boden einen Kalibedarf hat. Da aber der Phosphatbedarf viel grösser ist und bei KCl-Zusatz noch ausgeprägter wird, kann eine Kalidüngung wegen der durch dieselbe verursachten Steigerung des Phosphatmangels nur eine Depression der Ernte veranlassen.

Wenn aber genügend Phosphorsäure vorhanden ist, wird die Kalidüngung nur den Mangel an Kali vermindern und aufheben, was zu einer Ertragssteigerung führt. Je mehr Phosphorsäure im Boden zur Verfügung steht, desto mehr Kali scheint die Gerste ausnutzen zu können. Es liegt also eine

Tabelle 15.

Kornertrag des Versuches 12/1939. g Trockensubstanz
pro Gefäss.

Phosphat- düngung g	K a l i d ü n g u n g g					Durch- schnitt
	0	2	4	6	10	
0	15,19	9,40	5,73	4,37	1,47	7,2
2	29,60	31,10	29,56	21,15	14,37	25,2
4	35,87	40,20	36,62	31,92	14,96	31,9
6	38,77	43,13	42,74	44,91	28,04	39,5
8	29,82	44,65	46,61	45,14	35,30	40,3
15	42,20	44,08	41,81	43,88	42,51	42,9
Durchschnitt	31,9	35,4	33,9	31,9	22,8	31,2

Tendenz vor, dass das Maximum der Kurven mit steigender Superphosphatgabe nach rechts verschoben wird.

Dass man bei den höheren Kaligaben nicht mit einer Vergiftung durch Überdosierung zu tun hat, wird z. B. beim Studium der Kolumnen für 10 g Kalisalz in den beiden Tabellen klar. In jedem dieser beiden Fälle bekommt man bei Steigerung der Phosphatdüngung eine enorme Steigerung des Ernteertrages. Bei der höchsten Phosphorsäuregabe liegt der Ertrag auch in dieser Kolumne in beiden Versuchen weit über der durchschnittlichen Ernte.

Der mittlere Fehler der Differenz zwischen zwei Kolumnendurchschnittszahlen beträgt in Versuch 11 $\pm 3,05$ und in Versuch 12 $\pm 2,99$; und der zwischen zwei Reihen $\pm 3,34$ resp. $\pm 3,28$.

Kompletierende Bodenuntersuchungen.

Da die Erklärungen der hier beschriebenen Resultate auf der Theorie aufbauen, dass die Kalidüngung die Phosphatlöslichkeit in gewissen Böden vermindert und auf diese Weise einen Phosphatmangel verursachen kann, war es auch von

Interesse zu untersuchen, ob sich diese vermutete grössere Festlegung der Phosphorsäure bei KCl-Zusatz durch chemische Untersuchung des Bodens nachweisen liess. Zu diesem Zwecke wurde mit demselben Boden, der zu den Versuchen 11 und 12 benutzt worden war, folgende Untersuchung gemacht: Zu je 20 g des Bodens wurden 250 cm³ einer Lösung von KH_2PO_4 gegeben. Die so abgemessene Lösung enthielt 60 mg P_2O_5 und weiter KCl in steigenden Mengen. Diese Mengen waren 0, 0,2, 0,5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50 und 100 mg. Der Boden wurde dann mit diesen Lösungen während der Dauer von 302 Stunden, die auf 18 Tage verteilt waren, geschüttelt.

Da ein Vergleich mit den Gefässversuchen vielleicht wünschenswert ist, muss erwähnt werden, dass die hier benutzte P_2O_5 -Menge etwa fünfmal so gross war wie die höchste P_2O_5 -Gabe in den Gefässversuchen, und dass die benutzten Kalimengen ein etwas grösseres Gebiet umfassten, als die in den Gefässversuchen vorkommenden.

Nach der Schüttelung wurde die Flüssigkeit abfiltriert und die im Filtrat zurückgebliebene Phosphorsäure bestimmt. Es zeigte sich da, dass beinahe die ganze zugeführte Phosphatmenge vom Boden festgelegt worden und dass also nur eine kleinere Menge noch in der Lösung geblieben war.

Diagramm 8 zeigt die Resultate dieser Untersuchung. Auf der Ordinate sind die in der Lösung zurückgebliebenen P_2O_5 -Mengen in g aufgetragen worden und auf der Abszisse die Logarithmen der zugesetzten mg KCl. Die Kurve ist so ausgeglichen worden, dass jeder Punkt aus den sukzessiven Durchschnitten dreier neben einander liegender Werte bestimmt worden ist.

Dass die Phosphatlöslichkeit bei den Momenten abgenommen hat, bei denen die grössten Mengen KCl zugesetzt wurden, wird beim Blick auf die Kurve klar. Es scheint aber auch, als ob kleinere Mengen KCl eine kleine Erhöhung der Phosphatlöslichkeit veranlassen könnten. Dies könnte dann mit der Steigerung der Ernte durch mässige Kalidüngung, die aus den Versuchen 11 und 12/1939 sich ergab, in Zusammenhang stehen.

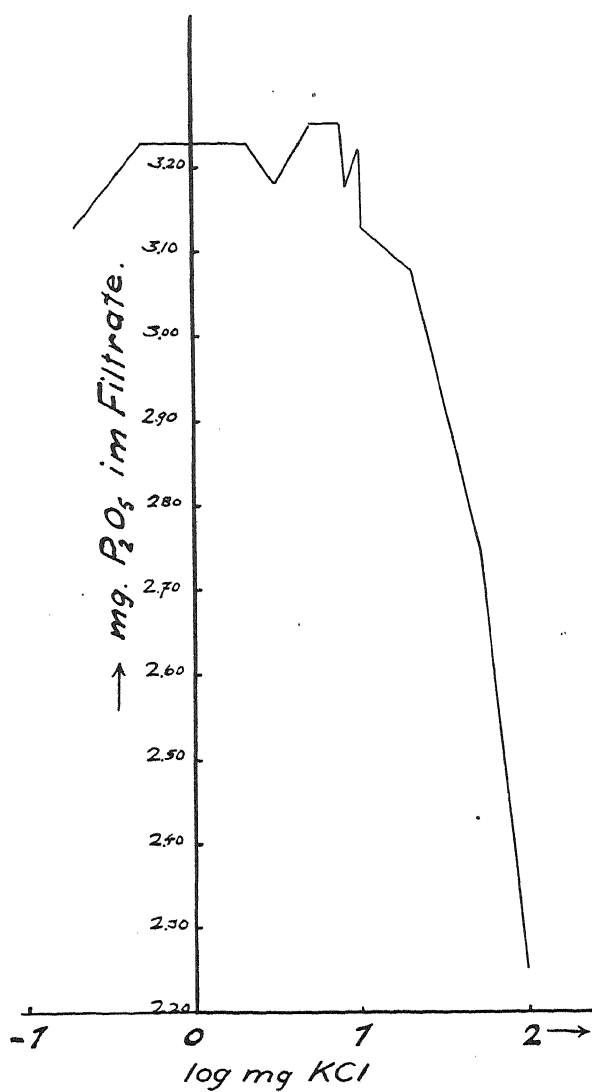


Diagramm 8.

Phosphatfestlegung im Boden bei steigendem KCl-Zusatz.

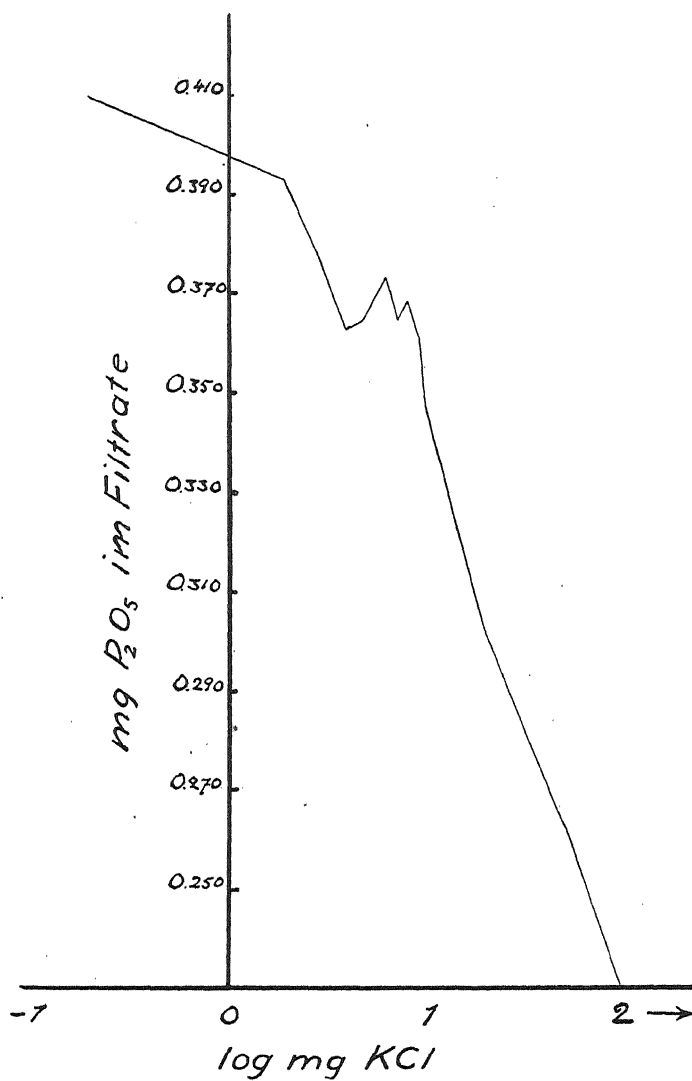


Diagramm 9.

Phosphatfestlegung im Boden bei steigendem KCl-Zusatz.

Die Kurven dieser Versuche (Diagramm 6 und 7) zeigten ja für höhere Phosphatgaben durchgehends Maxima bei Kaligaben, die etwa dem höchsten Verlauf der Kurve in Diagramm 8 entsprechen.

Dieselbe Untersuchung wurde auch an einem noch stärker phosphatfestlegenden, gyttjahaltigen Lehm Boden wiederholt, um zu sehen, wie sich dieser Boden verhält. Bei diesem Boden wurde die Phosphorsäure durchgehends noch vollständiger festgelegt, so dass nur ganz kleine Mengen in der Lösung zurückblieben. Die zugesetzte Quantität KH_2PO_4 war dieselbe wie im vorigen Experiment.

Die im Filtrat dieses Bodens vorhandene Phosphorsäure ergibt sich aus Diagramm 9. Auch in diesem Experiment ist es deutlich, dass weniger Phosphorsäure in der Lösung bleibt, wenn der KCl-Zusatz gesteigert wird. Der Knick der Kurve zwischen 4 und 10 mg KCl ist schwer zu erklären. Merkwürdigerweise zeigt die Kurve des Diagramms 8 bei denselben KCl-Mengen auch einen ähnlichen Knick.

Es scheint also, als ob die aufgestellte Theorie über die Einwirkung der Kalidüngung auf die Festlegung der Phosphorsäure durch die vorgenommenen Untersuchungen der Böden bestätigt würde.

Dies stimmt auch mit den Ansichten von DEMOLON und BASTISSE (1933 & 1934) überein, wenn sie schreiben: »La présence d'un anion inactif dans la solution de phosphate en contact avec le sol élève le taux de fixation de P_2O_5 ». In unserem Falle dürfte die Ursache der gesteigerten Festlegung also den Cl-Ionen zuzuschreiben sein. Dies lässt sich auch gut mit den vorher erwähnten Theorien von MATTSON vereinen.

Allgemeine Diskussion.

Welche Bedeutung haben nun diese Resultate für die praktische Düngerlehre? Darauf ist noch nicht leicht zu antworten. Die hier untersuchten Böden sind ja ziemlich auf gewisse Gegenden beschränkt und sind also nicht für schwedische Acker-

böden repräsentativ und noch weniger für Ackerböden im allgemeinen.

Der Verfasser hat deshalb die Feldversuche der lokalen Düngungsversuchstätigkeit Schwedens für die letzten Jahre studiert (SUNDELIN, LARSSON & ELIASSON 1933—1938), um zu ermitteln, wie die Ernte unter verschiedenen Umständen reagiert, wenn Kali zu einer vorher phosphatgedüngten Saat gegeben wird. Von sämtlich etwa 450 untersuchten Feldversuchen an Getreide auf Mineralböden in den Jahren 1932—1936 zeigen 45 % eine Ernteabnahme für »Kali- und phosphatgedüngt« gegenüber »Nur phosphatgedüngt«. Beim Versuch, dieses Material in Gruppen zu zerlegen, um diejenigen, die negativ reagieren, für sich zu bekommen, stösst man auf Schwierigkeiten. Das Material ist in den Versuchspublikationen so zusammengestellt, dass man es nach geographischer Lage, gebauter Pflanze und Reaktion, resp. Phosphatzustand des Bodens gruppieren kann, aber dass es nicht möglich ist, eine genügend feine Gruppierung nach Bodenarten durchzuführen.

Zweifelsohne ist aber gerade die letztgenannte in diesem Falle gefordert, da es in erster Linie von den qualitativen Eigenschaften des betreffenden Bodens abhängt, wie die Phosphorsäure festgelegt wird.

Es ist zu bedauern, dass es zur Zeit nicht möglich ist, das vorliegende Feldversuchsmaterial so zu bearbeiten, dass man eine endgültige Antwort auf die Frage bekommen kann, auf welchen Böden man mit gesteigerter Phosphatfestlegung bei Kalidüngung rechnen muss.

Es dürfte jedoch deutlich sein, dass die grosse Anzahl der Versuche, die nach Kalidüngung eine Ernteabnahme anstatt einer erwarteten Steigerung des Ertrages aufweisen, kein blosser Zufall ist, sondern dass man in mehreren Fällen mit bestimmten Eigenarten der Böden zu tun hat. Eine Art von Böden, die diesen negativen Effekt von Kali auf die Phosphatlöslichkeit zeigt, sind die in Mittelschweden gewöhnlichen gyttjahaltigen Mineralböden, die ziemlich viel Sesquioxiden enthalten.

Auch aus Dänemark liegen Versuchsergebnisse über negativen

Effekt für Kali- und Phosphorsäuredüngung gegenüber nur »Phosphatgedüngt« vor (BONDORFF 1938).

Die hier vorgebrachten Resultate und Gesichtspunkte über die Festlegung der Phosphorsäure im Boden bei Vorhandensein von Neutralsalzen, besonders von gewöhnlichem Kalisalz, sind natürlich nur ein Beitrag zur Erforschung des grossen Problems, wie die P- und K-düngemittel in verschiedenen Böden gegenseitig aufeinander einwirken. Es dürfte jedoch klar sein, dass, ehe man sich entschliesst, jemandem zu einer gleichzeitigen Phosphat-Kalidüngung zu raten, eine Erwägung der spezifischen Bodeneigenschaften am Platz ist. Unter schwedischen Verhältnissen dürften Böden mit relativ hohem Sesquioxidgehalt Neigung dazu haben, die Phosphorsäure bei Kalisalzzufuhr stärker festzulegen.

Zusammenfassung.

Die vorliegende Arbeit berichtet über Untersuchungen, die am Institut für allg. Ackerbaulehre an der landw. Hochschule Schwedens über die Einwirkung einer Kalidüngung auf die Festlegung von Phosphorsäure im Boden ausgeführt worden sind.

Die Untersuchungen sind in der Hauptsache auf einer Anzahl von verschiedenen Böden als Gefässversuche ausgeführt worden, die zum Teil durch Bodenanalysen ergänzt wurden. Es zeigte sich, dass auf Böden, die relativ reich an Sesquioxiden sind, die Phosphatfestlegung des Bodens durch Kalidüngung — 40 % Kalisalz — beträchtlich gesteigert wird, so dass die Pflanzen an Phosphatmangel zu leiden beginnen. Dieser durch Kalidüngung hervorgebrachte Phosphatmangel kann durch Steigerung der Phosphorsäuredüngung aufgehoben werden. Die Resultate der Gefässversuche werden durch die Bodenuntersuchungen bestätigt. Diese zeigen, dass bei Steigerung des KCl-Zusatzes zum Boden die Phosphatlöslichkeit abnimmt. Die Theorie wird lanciert, dass die durch Neutralsalzzusatz aktivierten Eisen- und Aluminiumverbindungen des Bodens die Phosphorsäure festlegen.

Es wird nachdrücklich hervorgehoben, dass verhältnismässig viele der schwedischen Feldversuche eine negative Wirkung des Kalis als Zusatz zu Phosphatdüngung zeigen und dass man eine Kalidüngung auf phosphatarmen Böden nicht ohne Berücksichtigung der Bodenbeschaffenheit empfehlen kann.

Sammanfattning.

Arbetets titel: *Om kaligödslingens inverkan på fosforsyrans fastläggning i jorden.*

Föreliggande arbete redogör för undersökningar, som utförts vid lantbrukshögskolans institution för allmän jordbrukslära, över den inverkan som kaligödsling i vissa fall har på fastläggningen av fosforsyra i jorden.

Undersökningarna ha huvudsakligen utförts som kärlförsök, vilka delvis kompletterats med kemiska laboratorieundersökningar. Företrädesvis har arbetats med de i Mellansverige rätt vanliga gyttejelerorna. Det visar sig, att på jordar, som äro relativt rika på seskvioxider, fosfatfastläggningen i jorden betydligt ökas genom gödsling med 40 %-igt kalisalt, och att växterna sålunda komma att lida av fosfatbrist. En dylik, genom kaligödsling framkallad fosfatbrist, kan upphävas genom stegring av fosforsyregödslingen.

Dessa resultat från två års kärlförsök bekräftas även genom de utförda laboratorieundersökningarna, vilka visa att fosforsyrans fastläggning ökar vid successiv KCl-tillsats till ifrågasvarande jord. Den teorien framlägges, att de genom neutral-salttillsatsen aktiverade järn- och aluminiumföreningarna i jorden fastlägga fosforsyran.

Vidare riktas uppmärksamheten på att ett förhållandevis stort antal av de svenska fältförsöken visa negativ verkan för fosfat- plus kaligödslat jämfört med enbart fosfatgödslat, och att detta icke torde bero på någon slump. Utan att först ha beaktat jordens beskaffenhet torde man ej böra rekommendera en kaligödsling på fosfatfattiga jordar.

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Studies on the relation between H-ion concentration and nitrate utilization by wheat plants.

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From the Institute of Plant Physiology.

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1. Introduction.

The influence of the H-ion concentration of the nutrient medium on the absorption and assimilation of different kinds of nitrogen by the plant has been a subject frequently dealt with in the literature. The reaction is often supposed to exert a dominating influence on the different utilization of ammo-

nium and nitrate nitrogen. The results of earlier research work on the subject, which have been reviewed in contemporaneous works by Loo (1931) and PIRSCHLE (1931), can briefly be summarized as follows: an acid or weakly acid medium has proved to be most favourable for nitrate nutrition and an alkaline reaction will promote the absorption of ammonium. Some recent works will be reviewed below; only those have been selected, however, which deal with the problem more in detail or have revealed new aspects of it.

In most experiments only a few different H-ion concentrations have been compared. Loo (1931) has carried out extensive water culture experiments, in which the whole pH range from 3 to 8 (or 9) was covered in close intervals. In experiments of short duration (6 to 48 hrs) the intake of NO_3 and NH_3 nitrogen by corn seedlings was studied and, on the whole, the earlier results were confirmed, i. e. the optimum for NO_3 -absorption was found at a weakly acid reaction. In fact Loo found not only one optimum but several, separated by distinct minima at pH values of approximately 5.2, 6.4, 7.0—7.2, and 7.8—7.9. This was explained by assuming several IEP:s of the cell plasm, where the absorption ought to be retarded.

PIRSCHLE (1931) has attacked the problem from quite another point of view. Several plant species were cultivated for some weeks in flowing nutrient solutions of varied reactions. At the end of the experiments the plants were harvested, the dry weights determined, and the material analyzed for nitrogen. In nitrate solutions a growth-optimum was obtained at pH 5 (approximately). A pH value of 3 proved to be toxic and the growth of e. g. most cereals decreased also at alkaline reaction. For some other plants, on the contrary, a growth minimum appeared at pH 7 followed by a rise at alkaline reaction. PIRSCHLE rightly emphasizes, however, that »zweigipfelige Wachstumskurven keine allgemeine gültige Bedeutung im Sinne artspezifischer Eigenschaften haben, sondern nur im Rahmen der jeweiligen Bedingungen gelten». He has also called atten-

tion to the probable relation of the growth minimum at pH 7 to the common occurrence of chlorosis at this reaction. Furthermore, PIRSCHLE explains his pH curves by a rise in the permeability caused by the H and OH ions at strongly acid and alkaline reactions. According to this view an excessive intake of NO_3 or NH_3 respectively should exert a toxic influence upon the plants; the action of nitrate was supposed to be less severe than that of ammonium. On the other hand, PIRSCHLE concluded from the determinations of the nitrogen contents of the plants that a direct relationship of the reaction of the nutrient medium to the nitrate absorption could not be traced. This was said to contradict the usual opinion of the optimal intake at a weakly acid reaction.

In a series of papers SHIVE and collaborators (CLARK & SHIVE 1934 *a, b*, DAVIDSON & SHIVE 1934, ARRINGTON & SHIVE 1935) have compared the influence of acid reaction (pH 4) with that of neutral (pH 7 or 6—8) on nitrate and ammonium absorption. Their experimental technique forms in some respects a combination of those of LOO and PIRSCHLE: plants were cultivated for some time in different nutrient solutions and at regular intervals the rate of ion absorption was determined by short time tests (6 hrs to a few days). This method was inaugurated earlier by HOAGLAND (1923). The results agree well with those of earlier investigations in as much as pH 4 was more favourable for nitrate and pH 7 for ammonium absorption. CLARK & SHIVE (1934 *a, b*) furthermore emphasize the fact that the nitrate content of the plants is independent of the rate of absorption. They conclude that the absorption and assimilation have their optima at the same reaction, and the latter is said to be directly regulated by the reaction of the nutrient medium.

BÜNNING (1936) and ITZEROTT (1936) could not confirm these results, nor could they find a direct influence of the reaction on the rate of intake of nitrate by cells. They conclude that the accumulation of nitrate within the cells depends upon the reaction outside: at a pH below 3, NO_3 is accumulated,

while at a higher pH it is excreted from the cells into the surrounding medium. The accumulation is supposed to depend upon the absorbing power of cell sap colloids. The plant material used in these experiments was *Aspergillus*, which differs, however, in certain respects from higher plants. Unicellular organisms have a very small accumulating volume in comparison with their large absorbing surface, a fact which might be of importance in determining the capacity for accumulation.

It is evident that the influence of the H-ion concentration upon the nitrate utilization has been explained as an influence upon absorption, assimilation, or accumulation of nitrate. It is also evident, however, at a closer examination, that the experimental results of the cited works do not differ very much, at least they are not absolutely inconsistent. The main difference lies in the different experimental techniques used, different points of view, and especially in a rather confusing terminology.

In the works of LOO and SHIVE absorption means the amount of nitrate taken in by a plant of defined condition per unit time, e.g. the rate of absorption. PIRSCHLE has calculated the absorption (Aufnahme) as the nitrogen content of the plant at an arbitrarily chosen point of time, regardless of the fact that the nitrogen content on a weight basis might fluctuate during the course of development as does the rate of absorption. The same content of an element can be obtained either through decreased growth rate or increased rate of absorption, or vice versa. — The term absorption is often used in this sense, or as denoting the total amount of a substance taken in by the plant during the growth cycle. The rate of intake of a substance by the roots is sometimes called permeability, a term which ought not to be used in connection with absorption of ions. — The accumulation (Speicherung) of BÜNNING and ITZEROTT corresponds partly — but not wholly — to the absorption of PIRSCHLE. SHIVE on the other hand, has used the term accumulation in its most common meaning, strictly applying it only to the accumulation of unreduced nitrate, and the term assimilation for the reduction of nitrate and its transformation to organic N. ITZEROTT has apparently used assimilation as synonymous with absorption, and the assimilation of SHIVE she has called reduction.

If a uniform terminology is used, the main results of the cited works may be summarized as follows: the rate of absorption of nitrate is obviously directly affected by the reaction of the nutrient medium, this relationship, however, has not

been elucidated in detail. According to SHIVE the rate of nitrate reduction within the roots depends upon the external H-ion concentration. Whether this holds true also for the accumulation of unreduced nitrate seems doubtful, the results of SHIVE and from BÜNNING's school apparently contradict each other. As pointed out before their plant materials are, however, very different, and a comparison, therefore, hardly possible. The total nitrogen content of a mature plant, finally, can scarcely be interpreted correctly in terms of nitrogen utilization, the complicated process of growth also being involved in regulating the nitrogen content within the plant.

The object of the present investigation was to study on one plant material — wheat in water cultures — the influence of the H-ion concentration on the absorption of nitrate, its reduction within the plant, and the accumulation of unreduced nitrate.

The following terms have been used. Assimilation denotes the reduction of nitrate and its transformation into organic N, in wheat roots these two reactions follow each other immediately, so that they might be regarded as one physiological process. Accumulation is used only with regard to unreduced nitrate, and absorption for the rate of entrance of nitrate into the roots.

In order to study the influence of the H-ion concentration upon the rate of intake of one ion — i. e. the effectiveness of the mechanism for the active ion absorption — it is necessary to follow the absorption by a uniform plant material from solutions of constant composition and — as far as possible — with the pH as the only variable factor. The claims are self-evident, yet they are impossible to fulfil, either practically or theoretically.

The claim to a uniform plant material implies short time experiments. If plants are cultivated for a long time in solutions of different reactions, the total absorption of all ions will become different, the plants will change their initial characters and the growth rates will become more or less unequal. In such a case the intake of a special ion is no longer due only to

the differences in pH but also to the qualitative and quantitative changes within the plant itself. In experiments of long duration it will become impossible to distinguish between direct effects of the H-ion concentration and indirect ones. This remark holds true for all cases where the influence of environmental factors upon a special phase of plant nutrition is to be studied. The duration of the absorption tests therefore must be made as short as possible; for that reason it is impossible to determine the absorption from the increase of nitrogen within the plant. This has to be calculated from the decrease of nitrate concentration in the nutrient solution.

A constant reaction of the nutrient solution can be obtained either by taking a large volume of solution, e.g. flowing solution, or by having it strongly buffered. On the other hand, if the nitrate absorption is to be determined from the decreased nitrate content of the solution, it is necessary that it amounts to at least 20—25 % of the initial concentration, if the analytical errors are not to become unsatisfactorily large. As a consequence, the reaction can not be held quite constant unless the buffering action is very strong or as the anions and cations are absorbed in equivalent amounts, which happens only occasionally. Too strongly buffered solutions are, however, not advisable, as they involve large and varying amounts of added salts; furthermore, one single buffer system is wholly effective only within a limited pH range. For that reason slight fluctuations of pH are inevitable in an otherwise satisfactory experimental technique.

The most suitable method of obtaining approximately constant reaction is working with flowing nutrient solutions. On the other hand, experiments on nitrate absorption imply aseptic work. Arranging flowing cultures aseptically is technically possible but involves a rather unwieldy technique. This difficulty has been avoided by making two kinds of experiments: one with flowing solutions, ordinary non-aseptic plants and very constant reactions, another with fully aseptic plants, stagnant solutions, and, consequently, a somewhat less constant pH. As in the main identical results have been obtained with both methods, a rather high significance can be attributed to them. Some further tests were made with non-aseptic plants of the same kind as in the flowing cultures but with solutions as in the aseptic experiments.

2. Experiments with flowing nutrient solutions.

Methods.

The experiments with flowing solutions were arranged as outlined in fig. 1.

A 2-liter stock flask was connected by means of siphons with two culture vessels of 75 ml volume. These were closed with rubber stoppers supporting cork discs of earlier described construction (cf LUNDEGÅRDH 1932 for full particulars concerning the culture technique), each carrying the root systems of 14 wheat plants, aged 16 to 19 days. These had been grown in complete nutrient solutions until the start of the experiment, when the aerial parts were cut off and the discs with the roots transferred to the experimental vessels. A branching tube on each vessel served as an outflow for the solution, the level of which was kept some mm beneath the cork disc, the bases of the stems not coming in contact with the solution. Thus the root systems were in normal position; the mere removing of the green parts does not affect the ion absorption of grown up plants for a short time. The rate of flow of the solutions was regulated by means of $\frac{1}{20}$ mm capillaries inserted in the siphons. The duration of the experiments was 22 hrs, during which time about 820 ml solution passed over the roots. The difference in level between stock flask and experimental vessel was about 48 cm at the start and decreased during the course of the experiment to about 37 cm; the rate of flow was thus diminished by about 25 %. By increasing the difference in height this error could have been diminished, but being of minor

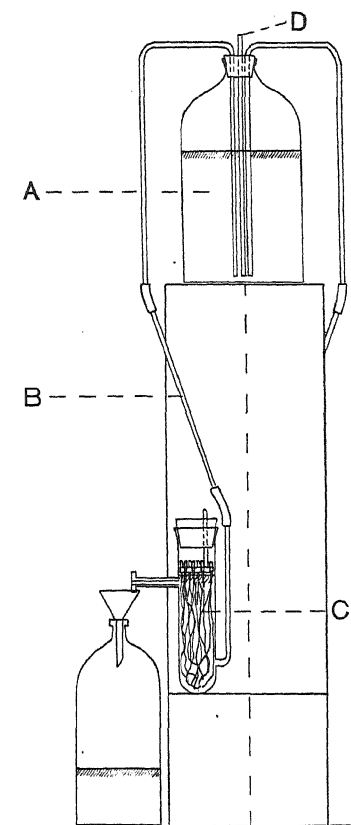


Fig. 1. Arrangement of experiments with flowing solutions. — A: stock solution, B: siphon with capillary inserted to regulate the rate of flow, C: experimental vessel with rubber stopper supporting root systems, D: tubing for compressed air.

importance this improvement was omitted. The stock solution was stirred and kept saturated with air by bubbling compressed air through it. The experiments were carried out at room temperature, $\approx 20^\circ$ C. Six duplicated experiments were run parallel.

The plants had been raised in large volumes of common nutrient solution with NO_3 as the source of N and at a pH of 5.5. This has proved to be the optimal reaction for the development of wheat plants. By changing the nitrate content of the solutions one day before they were taken for tests, roots with varied internal NO_3 contents could be obtained. The amount of nitrate per 28 plants (duplicates joint) ranged from 0 to 0.200 mmol. The weight of the roots amounting to about 6 g, the internal nitrate concentration on a green weight basis varied from 0 to 0.045 mmol per g.

The test solutions contained potassium-phosphate buffers of pH 3.2 to 7.7 with a constant P-concentration of $1/500$ -mol. In some series K_2SO_4 was added in order to make the K-concentration also constant. This did not, however, affect the results. Nitrate was added as KNO_3 at a concentration of $1/2000$ to $3/2000$ -mol.

Absolutely constant reactions could not be obtained. As pointed out before, this is theoretically impossible, if the decrease in the nitrate content of the solutions shall become measurable. The fluctuations were, nevertheless, very small and of no importance. On typical example will be given:

Initial pH:	3.2	3.9	4.8	6.1	6.8	7.5
Final pH:	3.3	4.1	5.3	6.1	6.8	7.4

A variation up to 0.2 pH units might be negligible. Larger deviations were found only within the pH range 4.5—5.0, owing to the fact that at this reaction the 1st H-ion of phosphoric acid is fully dissociated and the 2nd almost undissociated, the buffering action thus being nearly nil. In the diagram below, the points are plotted against the arithmetic mean of the initial and final pH.

In non-aseptic and not specially protected water cultures the roots will always become more or less infected by bacteria. As will be shown below by a comparison with aseptic experiments, this infection has had no visible effect on the results of the tests with flowing solutions. — Several experiments were specially carried out, however, to try the method suggested by GERRETSEN (1937), involving the arrangement of antiseptic cultures by the addition of Germisan to the nutrient solutions. The good results of GER-

RETSEN, however, could not be verified with our plant material. As a matter of fact, the wheat roots are surprisingly resistant even though grown for several weeks in solutions containing Germisan (up to 5 mg per liter). The stronger solutions recommended by GERRETSEN were, however, distinctly toxic, and lower concentrations had only imperfect bactericide action, as was shown by inoculations on agar.

The analyses for nitrate on solutions and plant material were performed according to methods described in detail earlier (BURSTRÖM 1938, 1939 *a*).

Results.

In all, 9 series of experiments of this kind were carried out; their results agreed very well. The data for four of them are given graphically in figs 2 and 3. They have been marked A—D, their main difference being an unequal initial nitrate concentration of the roots. In A the plants had been kept in distilled water 2 days before the start of the experiment, the nitrate content of the roots thus being decreased to zero. In B the NO_3 content per 28 plants was 0.050 mmol, in C 0.090, and in D 0.204 mmol. B and C represent plants of normal composition, those in D were extraordinarily rich in nitrate.

Fig. 2 shows the relation between pH and the total amount of nitrate absorbed. From very low values at pH 3—4 the curves rise to an optimum between pH 5 and 6. Within the range of physiologically normal H-ion concentration, at pH 4 to 7.5, the curves are very flat, with deviations from the average of only $\pm 20\%$. An optimum intake at pH about 5.5 is indicated and a pH < 4 acts injurious. In some series more acid solutions were tested; at pH near 3 still lower values were obtained, the nitrate absorption decreasing to almost zero at this reaction. It seems highly probable, that this is caused by the toxic action of the H-ions, especially considering the experience of LUNDEGÅRDH (1938) that at pH 3 the root surface is entirely saturated with H-ions, the metallic cations not being able to replace them. Thus the surface of the plasm loses its normal constitution and might be unable

to function normally at all. The decrease in nitrate absorption at strongly acid reaction is consequently not due to a specific action of the H-ion concentration on the mechanism of nitrate absorption but to an irreversible destruction of the plasm surface.

In series D a very low absorption was observed owing to the high internal nitrate concentration of the roots which

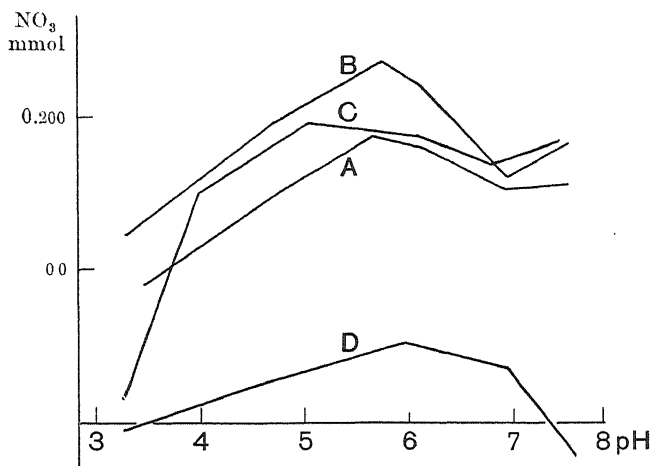


Fig. 2. The relation between pH and nitrate absorption by wheat plants in flowing solutions. Initial nitrate contents of the roots: curve A: 0, B: 0.050, C: 0.090 and D: 0.204 mmol.

impedes the absorption. The main course of the curve is, however, the same as in the other series with a flat optimum about pH 6. At pH < 4 and > 7 measurable quantities of nitrate were excreted into the external solution.

The absorbed nitrate is only in part assimilated by the roots, the rest being found as accumulated NO_3 at the end of the experiments. Fig. 3 shows the relation between pH and assimilated and accumulated nitrate, respectively, in the same four series as shown in fig. 2. The sum of the assimilated + accumulated nitrate does not, however, equal the values for ab-

sorption given in fig. 2, the amounts of nitrate initially present in the roots having to be added to the latter figures.

The pH-assimilation curves are of a very characteristic shape. The assimilation reaches a maximum at pH 6 and decreases

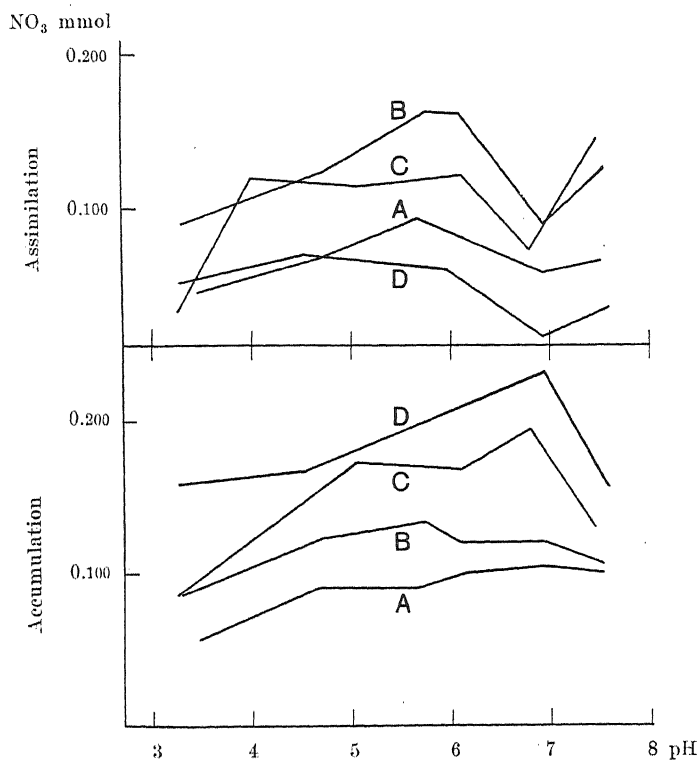


Fig. 3. The nitrate accumulation and assimilation in the same four series as in fig. 2.

with increased acidity similar to the absorption. The cause might also be the same. At pH 7 another minimum is found and from there the assimilation rises again as a weakly alkaline reaction is approached. The assimilation curves thus show two maxima and two minima; a third less interesting minimum will be found, of course, at strongly

alkaline reaction — at $\text{pH} > 8$ — owing to the injurious action of OH -ions.

The curves strikingly resemble the growth curves which show minima at $\text{pH} 7$, as obtained in some cases by PIRSCHLE (1931). The cause of the assimilation minimum at $\text{pH} 7$ will be discussed in detail further below; only certain points will be emphasized here.

In isolated root systems or intact wheat plants aged 2 to 3 weeks the absorption of nitrate is, on the whole, independent of the rate of assimilation within the roots (BURSTRÖM 1938). That is to say, a decrease in the assimilation will cause an increased accumulation of unreduced nitrate without affecting the absorption, at least not for a short time. The assimilation, on the contrary, depends upon the rate of absorption: decreased absorption will cause a decrease in assimilation because of the diminished amount of nitrate available within the roots.

This fact is related to the large capacity for accumulation in comparison with the relatively slow absorption which apparently takes place in only a small segment of the root (BURSTRÖM 1939 b), as does also the bulk of the assimilation. Accumulation, on the other hand, is found in all parts of the root system. The total amount of nitrate absorbed by the roots depends upon the composition of the nutrient solution and the mechanism for active ion absorption, but it does not depend upon the intensity of nitrate assimilation or the capacity for accumulation, providing the latter does not exceed a certain limit. — In roots of very young seedlings other conditions prevail, as will be discussed below.

According to earlier results (BURSTRÖM 1938) the assimilation by intact wheat roots at $\text{pH} 5$ to 6 amounts to about 50 % of the nitrate made available within the roots. This is also the case in the present experiments within the pH range 3 to 6 ; the decreased assimilation at $\text{pH} < 4$ is due to the decreased absorption. The second minimum at $\text{pH} 7$, however, finds no equivalent in the absorption. At this reaction the assimilation is either directly retarded by the external H -ion

concentration or it is indirectly influenced by some other factor than the rate of absorption.

In fact an insignificant minimum at pH 7 is indicated also in the absorption, but it is nevertheless obvious, that the very marked depression of assimilation can not be caused by the slightly reduced absorption, especially as the accumulation has its maximum even at this point. It is, on the contrary, probable that such strong fluctuations in the rate of assimilation as are found in the pH range 6 to 8 must cause changes also in the amounts absorbed, even if under more normal conditions the absorption seems to be independent of the assimilation. Considering this, it is very likely, that the absorption is still less directly affected by the reaction than might be believed from the curves in fig. 2. This question will be further discussed below.

The accumulation, finally, only consists of the difference between the amounts absorbed and assimilated as is clearly shown by the opposite shape of the curves in fig. 3. At low or medium internal nitrate concentration there is no reason for assuming a direct relationship between the pH and accumulation of unreduced nitrate. There is only one distinct exception from this rule; at very high internal NO_3 -concentration there appears, as pointed out before, an excretion of nitrate in the most acid and alkaline solutions. This fact could be expressed in the terms used by BÜNNING (1936) and ITZEROTT (1936) that above a certain reaction nitrate is accumulated, while below this reaction it is given off from the plant. That the relations in our experiments at pH 3 are quite the reverse of those of BÜNNING might be a matter of minor importance considering the large differences in the nature of the plant materials. This phenomenon does not appear, however, until the roots have reached a stage of saturation with nitrate. That the ion accumulation of the roots has its limits is easily shown with young excised roots possessing a rather low capacity for accumulation (BURSTRÖM 1939 b). That the excretion in our case takes place only at strongly acid reaction and at

the most alkaline reaction tested must be a matter connected with the very slow absorption at these reactions and has probably the same cause. The increasing disorganization of the plasm surface, due to the disturbed ion balance, will diminish the power of retaining the nitrate as well as lower the rate of absorption.

It has already been emphasized that the nitrate absorption is very nearly independent of the internal nitrate content of the roots; only in series D is the absorption retarded by the very high internal nitrate concentration (fig. 2). In fact the absorption proceeds so slowly in this series that it is hardly sufficient to compensate for the nitrate consumed in assimilation. The accumulation will decrease somewhat below the initial level, except at pH 7, where the assimilation is retarded. The roots are obviously saturated with nitrate and the slow absorption which takes place to compensate for assimilation or excretion due to unfavourable conditions will give a very incomplete picture of the normal absorption. The accumulation in this case is fluctuating about a semi-static state and the absorption under normal conditions is a decidedly dynamical process. It is thus evident that plant material characterized by a strongly inhibited absorption is very little apt for studying the mechanism of active ion absorption. In this connection there may be mentioned unicellular organisms which are rapidly saturated with salts to a true or apparent equilibrium, and isolated organs forced to a normally not occurring absorption of ions. It seems doubtful whether it is correct to apply the conclusions from such experiments to an explanation of the normal mechanism of absorption. This must be studied as a dynamical reaction of organs capable of continuous and intensive absorption. That this claim is justified will be made clear through the discussion of the absorption of excised roots (paragraph 4).

3. Experiments with non-aseptic plants in stagnant solutions.

In recent works on the physiological importance of the reaction of the nutrient medium, it has often been emphasized that a definite pH optimum for such a physiological process as growth does not exist, the position of the optimum being influenced by the nutritional state in general (PIRSCHLE 1931, ÅSLANDER 1935, SOLBERG 1935). Special stress has been laid upon the fact that the total salt concentration seems to be of

more importance for the development of plants than the H-ion concentration.

The more complicated a physiological process, the less chance of finding a simple definite relation to an environmental factor. On the other hand, for a less complicated process, it is more probable that such a relation might be traced. The highly complicated process of growth is due among other things, to the absorption of at least 10 different nutrient elements from the surrounding medium. Even if the absorption of each element stands in a fixed relation to the H-ion concentration, the relation to the reaction of the total absorption of all elements, as manifested by growth, must be less clear and affected by the balance of all ions etc.

From the absence of a definite growth-pH relation it must not be concluded that such a relation does not exist for the absorption of each element. It was necessary to prove the validity of the found pH curves, especially to find out if the position of the optimal nitrate absorption was affected by the total ion concentration of the solutions.

A number of experiments were carried out with plants of the same kind as in the preceding series but with non-flowing solutions. The possibility of varying the composition of the solutions was greater with such an arrangement.

The tests were performed in tubes of 200 ml volume, each containing 14 plants. They were kept in a water bath at 20° C. The duration of the test was 24 hrs. The solutions were stirred and aerated by means of compressed air. Each series comprised 5 or 6 experiments, each experiment being duplicated 4 or 3 times respectively. The pH was varied between 3 and 7, more alkaline solutions not being used because of the acidifying action of the carbon dioxide produced in respiration.

Further experimental details were as follows: age of plants 14 to 21 days; potassium or sodium phosphate buffers $\frac{1}{500}$ - to $\frac{1}{100}$ -mol; nitrate added as K or Na nitrate at concentrations from $\frac{1}{500}$ - to $\frac{1}{200}$ -mol; volume of the test solutions 75 to 150 ml per 14 plants, the nitrate added thus ranging between 0.187 and 0.750 mmol; initial nitrate content of the roots 0 to 0.045 mmol per 14 plants (0 to 0.015 mmol per g green weight).

Thus the concentrations of the solutions were varied within wide limits — the nitrate concentration up to twenty times and the total salt concentration up to six times that of the series with flowing solutions. Of course the H-ion concentra-

tions were somewhat less constant in these experiments than in the earlier ones, especially between pH 4.5 and 5.

It seems hardly necessary to report in detail the results of these experiments as the effect of the reaction upon nitrate absorption was in all essentials the same as in the preceding series. A flat optimum of absorption occurred between pH 5 and 6. No displacement of the optimum could be observed. — The relations above pH 7 will be studied thoroughly in the following paragraph.

4. Experiments with aseptic seedlings.

Methods.

These series were carried out with seedlings aged 4 days, germinated under sterile conditions and tested in aseptic solutions. The technique used and the arrangements of the experiments were the same as described earlier (BURSTRÖM 1939 *a*, *b*). Some points only will be emphasized.

In most series excised roots cut off from the seedlings were used; only in a few experiments were intact plants, separated from the grains tested. The experiments were carried out in 100 ml flasks containing the roots of 20 plants in 20 ml of solution. The flasks were autoclaved in advance. Two kinds of buffers were used. Usually a mixture of mono- and dibasic (in some cases also tribasic) sodium phosphate at a total P-concentration of 1/200 up to 1/100-mol was employed, in some series this was replaced by a mixture of acetic acid, sodium acetate, and NaHCO_3 saturated with carbon dioxide. The buffering action of this mixture was rather low within the pH range 6 to 7, owing to the varying amount of free CO_2 ; without CO_2 , however, this buffer was useless, because of the tendency of the respired carbon dioxide to increase the acidity to about 6. This buffer was used in those cases when phosphoric acid had to be excluded.

Even with phosphoric acid as a buffering agent the constancy of the reaction was less satisfactory than with flowing solutions, the H-ion concentration showing a tendency to increase 0.2 to 0.3 pH units. An example will be given:

Initial pH:	6.0	6.5	7.0	7.5	8.0
Final pH:	5.7	6.2	6.8	7.2	7.5

Solutions more alkaline than pH 7.5 were particularly less constant due partly to the low buffering action of the phosphoric acid.

The duration of all experiments was 24 hrs.

Nitrate was added as KNO_3 at a concentration of $1/400$ -mol. All chemicals were highly purified in order to remove heavy metals, consequently manganese had to be added, the concentration being 10 mg MnSO_4 aq per liter. Furthermore, the solutions were made $1/100$ -mol with respect to glucose. The tests were performed in a water bath at 25°C . Five experiments in quadruplicates were run simultaneously. The results have been calculated in $\mu\text{mols NO}_3$ per 80 plants.

The main features of the nitrate absorption and assimilation by young seedlings have been thoroughly dealt with in an earlier paper (BURSTRÖM 1939 *b*). Excised roots will give an example of an experimental material not suitable for studies on the mechanism of ion absorption. Assimilation, on the contrary, proceeds normally after separating the roots from the aerial parts.

It was shown, for example, that the excised roots will rapidly become saturated with nitrate; the absorption is impeded if nitrate is not removed by assimilation within the roots. These roots, in certain respects, resemble the case discussed in the preceding paragraph, in which old roots had been overfed and saturated with nitrate. Another possibility, which is particularly realized in smaller segments of roots, is that even if the absorption is functioning normally, the roots lack the power of retaining the superfluous nitrate absorbed, it will be excreted again through the wound surface into the nutrient medium. In this connection it is worth referring to the root-pressure, studied recently on excised roots by WHITE (1938), which might offer an explanation of this phenomenon. In any case it was confirmed that excised roots assimilate nitrate almost as rapidly as intact plants but that the total amount of nitrate absorbed is no exact measure of the actual rate of absorption. The calculated »absorption» of excised roots should more correctly be termed »retention». This is true particularly for excised roots but holds to a certain extent also for intact very young seedlings. In most respects, however, these behave as mature plants, but the absorption seems to be slightly hampered by the rapidly increasing nitrate content of their roots.

The nitrate utilization of intact seedlings.

One experiment will be reported to settle the question of conformity of intact seedlings and grown-up plants with regard to their assimilation of nitrate (table 1). The H-ion concentration was varied between pH 3.5 and approximately 9. The table gives an account of the amounts of nitrate absorbed,

Table 1.

pH series with intact seedlings. Na-phosphate buffer.

pH	Nitrate μ mols per 40 seedlings		
	absorbed	accumulated	assimilated
3.5	12.6	4.0	8.6
4.8	33.0	22.3	10.7
5.5	28.2	14.7	13.5
6.2	37.8	21.3	16.5
7.0	24.5	16.6	7.9
8.0	29.4	15.4	14.0
\sim 9.0	19.5	13.9	5.6

accumulated, and assimilated. The figures for the absorption are somewhat more irregular than in the preceding series for reasons already quoted. It is evident, however, that the absorption decreases towards both acid and alkaline reaction and has an optimum about pH 6. No stress can be laid upon the irregularities, a smoothed curve having the same shape as that for flowing solutions. Concerning the assimilation, the agreement is still better. From the low values at pH 3.5 it rises to a maximum at pH 6.2, shows a second minimum about pH 7, which is followed by another rise on the alkaline side. The most alkaline solution with a pH of about 9 — not exactly determined — proved to be toxic.

It is interesting to note the very good conformity of these results with those obtained for flowing solutions. Apart from the small irregularities quoted above the results are identical, yet both the nature of the plant materials and the composition of the test solutions have been markedly different.

The decrease in nitrate absorption in the most acid solution is, as has already been emphasized, probably due to the destructive effect of the high H-ion concentration. The cause of the injurious action of alkaline solutions might be analogous. In several cases a decreased absorption — and assimilation — appeared even at a pH value somewhat below 8. In most cases, however, it occurred only at a still more alkaline reaction as in the cited example. Thus the position of the second maximum is not exactly fixed. This disagreement seems to be of minor importance; if we assume an injurious action of the high OH-ion concentration, this might be rather labile and sensitive to small changes in the conditions of the plants.

While it has been shown that the minimum of assimilation at pH 7 appears under most varying conditions, it must be noted that all series hitherto have been arranged with phosphoric acid as the buffering substance. Full evidence that the depression is caused directly by the concentration of the H-ions can only be obtained if the results can be reproduced with other buffering agents. In fact, however, the possibility of exchanging the phosphoric acid is very restricted because it is most important that no injurious substances are brought into the test solutions.

Among the ions present in a natural substrate, only two — apart from the phosphoric acid — are weak electrolytes and thus suitable as buffering substances: CO_2 and NH_3 . The latter is scarcely convenient as a buffer, because the maximal buffering action takes place at pH 8.7; carbon dioxide is too volatile for use with aerated cultures. Colloidal buffers as used by MEHLICH (1939) are not well adopted for exact studies on ion absorption. It was therefore found necessary to introduce a non-physiological compound and acetic acid was chosen, as being the least injurious, to complement the carbonic acid system.

The results of two series with HAc-CO_2 buffers are combined and shown in fig. 4. Both cover the pH range 4.7 to 7.7 and agree very well. The upper full-drawn curve indicates the total amounts of nitrate absorbed, the lower curve the assimilation, both having been drawn free-hand through the indi-

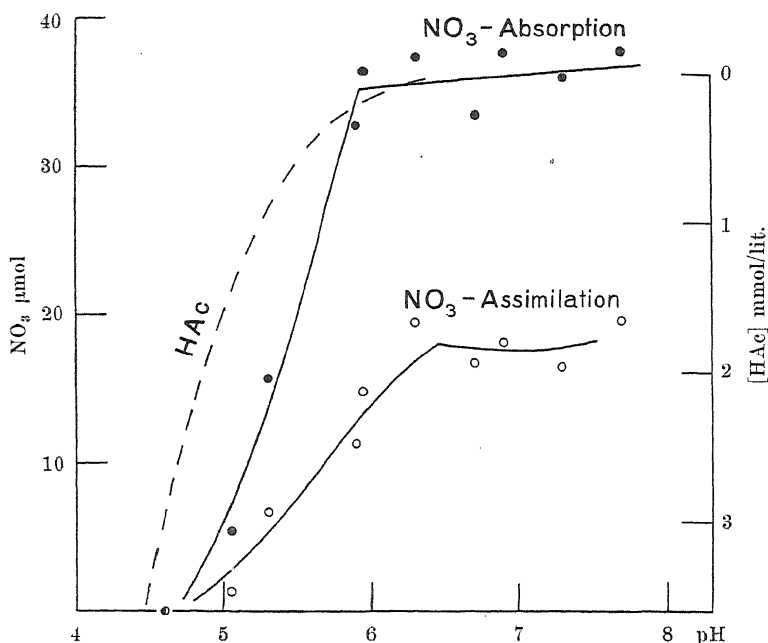


Fig. 4. The relation of pH to absorption and assimilation of nitrate by intact seedlings from acetate-carbonate buffers. — Black points indicate absorption, circles assimilation; scale to the left. — The dotted curve shows the concentration of undissociated acetic acid; scale to the right.

dividual points. The main course of the curves is the same, rising from zero at pH 4.5 to a maximum at pH 6 to 8. The assimilation on every point amounts to about 50 % of the absorption. It is quite evident that there is no sign of a second minimum at pH 7, instead of which the assimilation — and also the absorption — are markedly independent of the H^+ -ion concentration above pH 6.

The decrease in absorption from the maximum towards the acid side is much steeper than with phosphoric acid, reaching zero at pH 4.5 instead of at pH about 3. It seems highly probable that this is connected with the concentration of undissociated HAc in the solution, which is indicated by the dotted curve in fig. 4. This has been plotted arbitrarily in inverse position against the other curves, the point $\text{HAc} = 0$ corresponding to the maximum absorption of nitrate. Thus it seems that the decrease in absorption runs parallel to the increasing concentration of undissociated acetic acid. Nevertheless, it is not quite necessary to assume an injurious action of the rapidly penetrating acetic acid. It has been shown earlier (BURSTRÖM & BORATYŃSKI 1936) that wheat plants are able to endure 3 days in $\text{HAc } 1/200\text{-mol}$ without showing any signs of injury even at pH 3.8. It is possible that the decreased absorption is due to a mere hindrance of the ion absorption and not really connected with a damage of the roots. In any case, this part of the pH-curve is of minor importance for the present problems, as the action of acetic acid, though being theoretically interesting, has no bearing on the nitrate absorption under normal conditions. At a more alkaline reaction — pH > 6 — a specific action of the acetic acid is out of the question. At pH 7.2 wheat plants develop normally for several weeks in solutions containing $\text{Ac } 1/300\text{-mol}$ (BURSTRÖM & BORATYŃSKI 1936).

It might have been concluded therefore, that the depression at pH 7 is connected with the presence of phosphate in the substrate. In any case it is obvious that it is not merely due to an action on the part of the H-ion concentration.

On the other hand, phosphate and acetate buffers differ not only with respect to the nature of the anions but also with regard to the concentrations of the cations. All buffers hitherto used have been equimolar with regard to the anions, but as the phosphoric acid is much stronger than the acetic and carbonic acids, the cation concentrations are accordingly higher.

A $1/200$ -mol P-buffer, at pH 7 contains 7.8 mmols of Na per liter, whereas an Ac-CO₂-buffer of the same strength contains not more than 1.8 mmols. In order to finally settle the question of the difference between the buffer solutions it thus became necessary to re-investigate the action of the buffer concentration and the cations more exactly about pH 7.

The most convenient material for such series proved to be excised roots as their low power of absorption renders the reaction more constant.

The nitrate utilization of excised roots.

A number of series were carried out with excised roots in phosphate buffers within the pH range 6 to 8. Only one example will be discussed in detail (table 2).

Table 2.

pH series with excised roots. Na-phosphate buffer.

pH			Nitrate μ moles			Fresh weight g ¹
initial	final	average	absorbed	accumulated	assimilated	
6.0	5.8	5.9	50.4	30.2	20.2	0.92
6.5	6.5	6.5	30.9	24.6	6.3	0.90
7.0	6.7	6.9	15.8	15.8	0	0.83
7.5	7.3	7.4	23.6	17.0	6.6	0.87
8.0	7.5	7.7	33.4	22.2	11.2	0.90

The constancy of the H-ion concentration is somewhat better than the average (p. 146). At pH 6.7—7.0 (average 6.9) no assimilation takes place and hence it rises towards both acid and alkaline reactions, quite in accordance with the preceding results. The minimum is only more pronounced, the cause for this seems to be obvious. In intact

¹ Initial weight 0.77 g.

plants the assimilation takes place partly in connection with the absorption in the root hair zone, partly in the root base owing to the rich supply of carbohydrates from the grain, or, in grown-up plants to some extent in the leaves (BURSTRÖM 1938, 1939 *b*). The assimilation in the latter two cases is, of course, neither directly nor indirectly affected by the external H-ion concentration. In excised roots, on the contrary, the assimilation takes place solely in the youngest part of the root connected with the absorption, and thus the effect of the reaction becomes more marked.

In this series another phenomenon is met with, which sometimes appeared with excised roots. At pH 7 not only is the assimilation, but also the absorption, accumulation, and growth rate decreased. This is connected with the mentioned localization of the absorption. It takes place mainly in the youngest growing root hairs. If growth and development of new root hairs is promoted, the absorption and accumulation increase accordingly. If, on the other hand, growth ceases the absorption sooner or later must come to a stand-still because of the lack of functioning root hairs. A stronger nitrate assimilation sometimes induced an accelerated growth and thus increased the capacity for accumulation.

A rapid growth is, however, not always caused by rapid nitrate assimilation. Fig. 5 shows the relation of assimilation to growth rate in a number of experiments with excised roots. All points are distributed at random over the left upper half of the diagram above the dotted line. This means that a certain assimilation always promotes at least a certain increase in growth. The few values lying below the curve represent occasional irregularities of the plant material. It is also evident from the diagram that a fairly intensive growth is possible without nitrate assimilation; in these cases the accumulation shows correspondingly high values. One example is given in table 3. The assimilation decreases to zero at pH 7 the growth and accumulation, on the contrary, showing no minimum at this point.

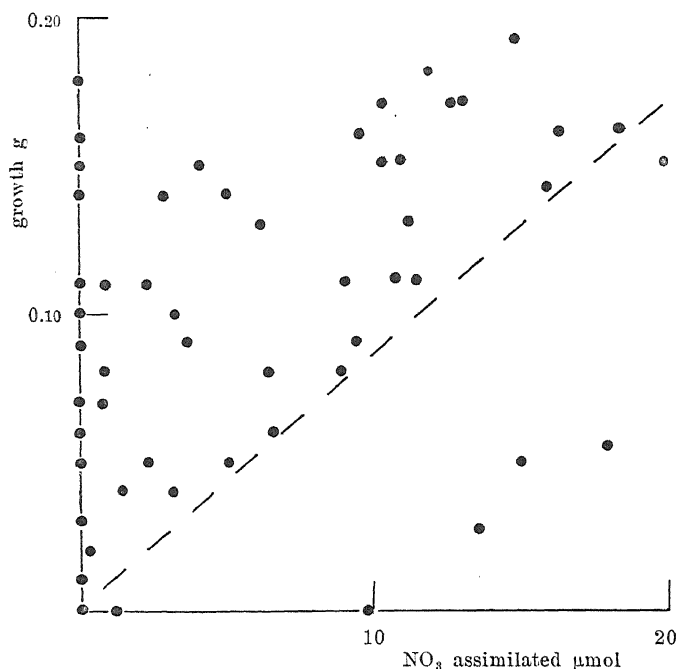


Fig. 5. The relation between nitrate assimilation and growth of excised roots.

Table 3.

pH series with excised roots. Na-phosphate buffer.

pH ¹	Nitrate μ mol			Fresh weight g ²
	absorbed	accumulated	assimilated	
6.0	31.7	16.8	14.9	0.80
6.5	32.1	28.9	3.2	0.80
7.0	25.8	22.1	3.7	0.79
7.3	23.5	12.0	11.5	0.81
7.5	26.3	11.2	15.1	0.75

¹ Average, as in all following series.

² Initial weight 0.70 g.

In consequence, the accumulation — or more correctly the retention — is very irregular, the rate of assimilation, however, is highly constant. A comparison of all series of this kind is made in fig. 6. It consists of 5 complete pH-series between pH 6 and 8 and some occasional values from other series.

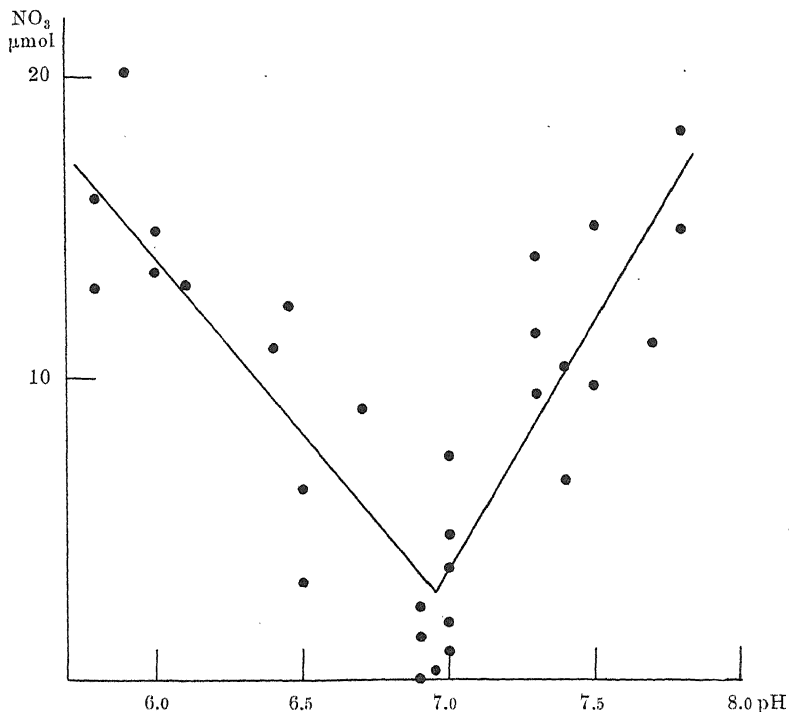


Fig. 6. The relation between pH and nitrate assimilation of excised roots.

The points group themselves smoothly around the mean curve, showing a sharply marked minimum of assimilation at pH 6.8 to 7, probably very near pH 6.9.

With acetate-carbonate buffers instead of phosphoric acid this minimum disappears completely in accordance with the preceding results. This is illustrated in table 4. From two series, one with PO₄ another with Ac buffers and which were otherwise identically the same, data have been compared at

pH about 6, 7, and 7.3. The depression of assimilation is clearly shown with the phosphate buffer but is totally absent with the acetate buffer. Even at optimal reaction, pH 6, the assimilation in acetate buffer exceeds that in phosphate and is quite constant within the pH range studied. It is also interesting to note that the accumulation is nearly constant in all solutions; the capacity of the roots for retaining the nitrate is not influenced by the nature of the buffer or the H-ion concentration.

Table 4.

Comparison between phosphate and acetate-carbonate buffers. Excised roots.

pH	Buffer	Nitrate μ moles		
		absorbed	accumulated	assimilated
5.9—6.0	{ P	42.4	29.4	13.0
	{ Ac	54.4	25.2	29.2
6.9—7.0	{ P	30.7	28.3	2.4
	{ Ac	60.8	28.0	32.8
7.2—7.4	{ P	43.9	29.8	14.1
	{ Ac	55.6	29.2	26.4

As pointed out earlier, the question then arises whether this difference between the buffer solutions is due to the nature of the anions or the concentrations of the accompanying cations. This was investigated by means of comparisons between phosphate and acetate buffers made equimolar with regard to the cation (Na) concentrations.

Dilution series with phosphate buffers are easily arranged, the buffering action being sufficient to prevent changes of reaction even at a concentration of $1/300$ -mol. With acetate-carbonate buffers it is more difficult to keep the reaction at a pre-determined value, especially between pH 6 and 7. For that reason these series were arranged with larger volumes of test solution

(25 ml) and a reduced number of plants (15 in each flask). It was thus made possible in two dilution series to keep the reaction fairly constant at pH about 7 with both phosphate and acetate buffers.

Each series contained 3 concentrations of phosphate buffer and 2 of acetate-carbonate. Being in all other respects equally arranged and agreeing with regard to the results, the series have been combined in one diagram

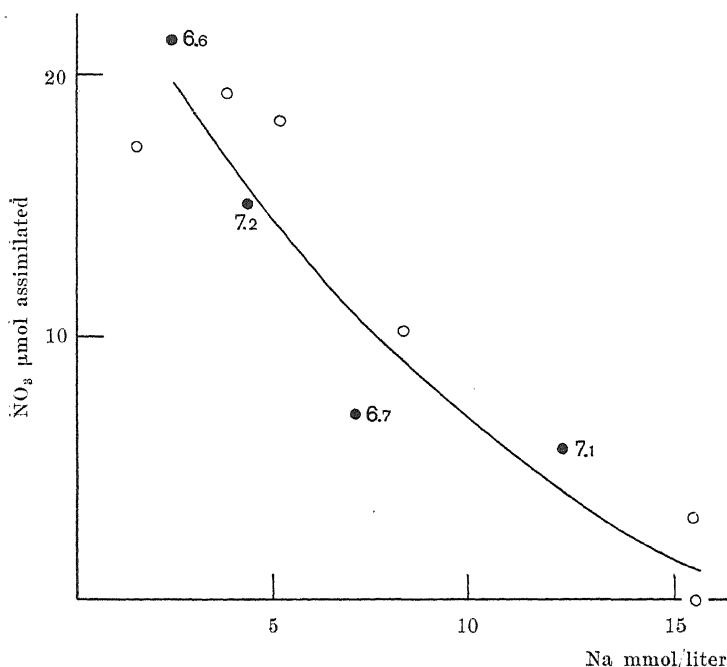


Fig. 7. The relation between sodium concentration and nitrate assimilation by excised roots at pH ~ 7 . — Circles indicate experiments with phosphate buffers of pH 6.9. Black points experiments with acetate-carbonate buffers, the pH of each being noted on the fig.

(fig. 7). On the abscissa the Na-concentration of the test solutions has been plotted, and on the ordinate the assimilation in μmols of nitrate, calculated per 80 plants to facilitate a comparison with preceding results. The circles indicate experiments with phosphate and the black points with acetate-carbonate buffers. In the former experiments, the average of the initial and final pH was 6.9; in the latter the reaction varied between 6.7 and 7.2, the value for each point being noted on the diagram.

Although the reactions of the acetate solutions are not quite in accordance with those of the phosphate solutions, the diagram clearly shows that the effect of the buffers on the assimilation is chiefly due to the Na-concentrations. That different results have been obtained with acetate and phosphate buffers seems to be a matter of the much higher Na-content of the latter. On the contrary, it has previously been emphasized that the total ion concentration of the nutrient solutions should not exert any visible influence on the main features of the pH-assimilation curve, at least not on the positions of the maximum at pH 5.5—6 and the minimum at pH 7. An explanation for this discrepancy must be found.

Cations and nitrate assimilation.

The influence of the cations on the nitrate assimilation has been dealt with in two earlier papers (BURSTRÖM 1939 *a*, *c*).

In the first paper it was shown, that presence of manganese is indispensable to nitrate assimilation by the roots; it was assumed that Mn is catalyzing some part of the assimilation process. In the second work the influence of other cations on the assimilation was studied in detail. It was confirmed that Mn can not be replaced by other cations. On the contrary, it was found that all other ions tested, including the alkali-, alkaliearth-, and heavy metals, decreased the assimilation by the roots even at rather low concentrations. A theory was advanced and also confirmed by the results. In its active form as a catalyst within the cell, manganese is in an unspecific way adsorbed to some colloidal plasm constituent, from which it can be easily exchanged by other cations and thus rendered inactive. Moreover an antagonistic decrease in the Mn absorption must be taken into consideration; this will appear, however, only at comparatively high concentrations of the added salts. The antagonistic effect on the uptake of manganese also being due to an exchange of ions, the retarding influence of other cations on the nitrate assimilation in general was explained by a replacement of adsorbed manganese.

It was likewise noted, that a manganese absorption less than about 0.30 μ moles per 80 plants per day was unsufficient for maximal rate of assimilation. If the absorption decreased below this approximate level, the assimilation was always diminished.

If, on the other hand, the manganese absorption amounted to 0.30 μ moles or more, the assimilation was higher or lower according to the concentrations of other cations present.

In mixtures containing three or more cations, this retarding effect was weakened, and in a complete, balanced solution an assimilation was obtained almost equalling that of a pure nitrate solution with only one cation present (except manganese). This was not, however, due to increased growth rate in the complete solution.

Table 5.

pH series in complete nutrient solution. Na-phosphate buffer with additions of $\frac{1}{1000}$ -mol CaSO_4 , $\frac{1}{2000}$ -mol MgSO_4 , Fe-citrate 10 mg per liter. Excised roots.

pH	Nitrate μ moles			Fresh weight g ¹
	absorbed	accumulated	assimilated	
6.2	37.7	22.8	14.9	0.95
6.6	31.5	20.7	10.8	0.90
7.0	29.8	19.4	10.4	0.94
7.5	48.0	29.4	18.6	0.95
7.7	53.3	29.9	23.4	0.95

These results were obtained in unbuffered solutions of pH about 5 or with phosphate buffers of pH 5.5.

The same phenomenon appears also at pH 7. It has been shown that an increase in the Na-concentration causes a decrease in the assimilation. — Table 5 exemplifies the assimilation from a complete nutrient solution. At pH 6 the assimilation is slightly lower than normal, at pH 7 a depression is visible, which amounts to only about 30 %. Subsequently the assimilation rises in alkaline solutions. The test solutions contained, as in the preceding series, Na-P-buffer, KNO_3 , and

¹ Initial weight 0.79 g.

Table 6.

pH series with Na-phosphate buffer and $\frac{1}{1000}$ -mol CaSO_4 .
Excised roots.

pH	Nitrate μmols		
	absorbed	accumulated	assimilated
6.2	41.4	36.3	5.1
6.6	31.0	28.7	2.3
7.0	36.5	36.3	0.2
7.4	29.5	28.7	0.8
7.8	23.0	21.8	1.2

Table 7.

pH series with Na-phosphate buffer and Fe-citrate 10 mg
per liter. Excised roots.

pH	Nitrate μmols		
	absorbed	accumulated	assimilated
5.8	49.1	33.1	16.0
6.4	46.8	35.8	11.0
6.9	25.6	24.2	1.4
7.4	51.4	41.0	10.4
7.8	34.4	31.3	3.1

Mn, to which had been added $\frac{1}{1000}$ -mol CaSO_4 , $\frac{1}{2000}$ -mol MgSO_4 , and 10 mg per liter Fe-citrate.

The question now arises as to whether this effect of the complete solution at pH 7 depends upon a specific action of one certain cation.

The action of $\frac{1}{1000}$ -mol Ca is shown in table 6. In this example the calcium has no influence upon the assimilation

at pH 7 and in more acid and alkaline solutions it acts only harmfully. The usual assimilation at pH 6 amounts to about 20 μ mols but it is reduced to 5.1 through the addition of Ca. The assimilation, in fact, approaches zero within the whole pH range in this case.

Iron, added as 10 mg per liter of Fe-citrate, has hardly any influence upon the assimilation when added by itself. This is shown by table 7. — This series also indicates an injurious action of OH-ions at a reaction slightly below pH 8.

The effect of $1/2000$ -mol MgSO_4 is illustrated by table 8. It is evident, that the assimilation in this case is not very different from that in a complete nutrient solution.

Table 8.

pH series with Na-phosphate buffer and $1/2000$ -mol MgSO_4 .
Excised roots.

pH	Nitrate μ mols		
	absorbed	accumulated	assimilated
5.9	62.3	42.4	19.9
6.4	50.4	35.4	15.0
6.9	44.0	33.0	11.0
7.3	58.8	35.4	23.4
7.7	46.9	30.5	16.4

Nevertheless, it would be too hasty an inference to attribute to magnesium a specific capacity for promoting nitrate assimilation at pH 7. Some series were arranged as shown in table 9. The nutrient salts were added successively until a complete solution was obtained. In this case the assimilation from the complete solution is about the same as in table 5, about 10 μ mols, and with only Na-P-buffer, KNO_3 and Mn zero. Evidently none of the ions added i. e. Fe, Ca, Mg, or Cu, is alone responsible for the improvement. As usual the highest

Table 9.

Na-phosphate buffer of pH 7.0 with additions of Ca, Mg, Fe and Cu. Excised roots.

Addition	Nitrate μ moles		
	absorbed	accumulated	assimilated
None	31.9	31.7	0.2
Ca	31.4	29.1	2.3
Ca+Mg	42.3	37.2	5.1
Ca+Mg+Fe . . .	41.0	34.5	6.5
Ca+Mg+Fe+Cu .	38.5	29.4	9.1

increase in assimilation was obtained with magnesium, but the other salts have also given noticeable effects. As it has already been shown that the decrease in assimilation is partly connected with the high Na-content, it is hardly probable that it should be specifically neutralized by Mg. It was also found in some experiments, not mentioned here in detail, that lower Ca-concentrations — about $1/3000$ -mol — had a much better effect than $1/1000$ -mol, in which concentration Ca entered the complete solution. Nevertheless it was even so inferior to Mg, which has its optimum about the tested concentration, $1/2000$ -mol. The reason for the strong action of Mg will be discussed below.

The cause of the difference between the assimilation at pH 5—6 and pH 7 is the fact, that in the latter case the assimilation is strongly inhibited by the presence of exclusively Na- or K-salts. The favourable action resulting from a balancing of the solutions by adding Ca-, Mg-, and other salts, is very similar in both cases.

It is thus obvious, that the depression at pH 7 is closely connected with the balance of the cations; that the H-ion concentration does not primarily

inhibit the assimilation is clearly shown by the following experiments.

A pH-series was carried out along the same lines as the preceding ones but without the addition of manganese (table 10). It will be seen that the assimilation is zero at all reactions. The differences between the amounts of nitrate absorbed and accumulated fall within the experimental errors. Nevertheless, a sharp minimum appears in the growth at pH 7 and thus also in the nitrate retention.

Table 10.

pH series with Na-phosphate buffer without manganese.
Excised roots.

pH	Nitrate μ moles			Fresh weight g ¹
	absorbed	accumulated	assimilated	
6.0	38.2	38.5	~ 0	0.94
6.5	25.6	26.9	~ 0	0.85
7.0	20.0	19.4	~ 0	0.79
7.4	27.0	27.0	~ 0	0.86
7.9	25.5	27.9	~ 0	0.87

By also excluding glucose from the substrate the growth is totally inhibited, the nitrate retention also showing low and constant values over the entire range of pH (table 11). In this series the K absorption was determined (spectroscopically, as also Mn in the following experiments). Though the values do not show the total absorption for reasons already quoted, it is interesting to note that even in this case the K retention has an obvious minimum at pH 7. This fact was confirmed by other series as well. It is thus evident, that even if no depression appears in such physiological func-

¹ Initial weight 0.80 g.

Table 11.

pH series with Na-phosphate buffer without manganese and glucose. Excised roots.

pH	Nitrate μ moles accumulated	K μ moles retained
6.0	24.5	8.0
6.5	18.7	-1.8
7.0	23.0	2.3
7.4	21.8	7.7
7.8	18.4	16.3

tions as growth, nitrate retention, or nitrate assimilation, it still appears in the capacity of the roots for cation retention.

More instructive than the values for K-retention are the results relative to the absorption of manganese. In the first place the manganese supply specifically regulates the nitrate assimilation and secondly the manganese retention apparently corresponds to the total amounts absorbed; because of the low mobility of this ion it is not so easily excreted again from the roots (cf. below).

Some examples of manganese absorption from unbalanced and balanced solutions are compared graphically in fig. 8. In unbalanced solutions the manganese absorption approaches zero at pH 6.5—7. The addition of only magnesium weakens the minimum at pH 7 somewhat, though it is still prominent, which means a slight but undoubtful promotion of the manganese absorption. In complete nutrient solutions quite another relation is found between pH and Mn absorption: the absorption increases rapidly and continually with decreasing H-ion concentration, and at pH > 7.5 all the Mn added is absorbed.

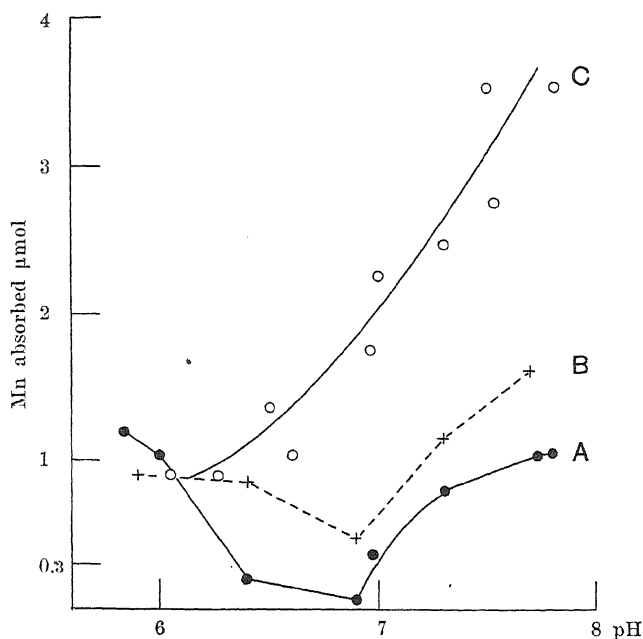


Fig. 8. The relation between pH and Mn-absorption by excised roots. — Curve A: unbalanced solutions, B: with additions of magnesium, C: complete nutrient solution.

This shows that the absorbed manganese is really retained by the roots and not excreted again in visible amounts.

It is of special interest to note that the same relation is found in complete solutions without nitrate (table 12). For the K retention, however, a depression at pH 7 is still noticeable.

The final evidence that the depression at pH 7 is connected with the cation absorption, is given in table 13. This shows the absorption of K and Cl from KCl solutions by intact seedlings at varied reactions. Within the pH range 4—7 the Cl absorption is independent of the H-ion concentration, which agrees with the results of the experiments with flowing solutions. In this case only a slightly indicated optimum-curve for the nitrate absorption was obtained, with

Table 12.

pH series in complete nutrient solution without nitrate. Composition of the solutions: $\frac{1}{800}$ -mol K_2SO_4 , $\frac{1}{1000}$ -mol $CaSO_4$, $\frac{1}{2000}$ -mol $MgSO_4$, Fe-citrate 10 mg per liter, $MnSO_4$ 10 mg per liter, $\frac{1}{400}$ -mol NH_4Cl , $\frac{1}{100}$ -mol Glucose. Excised roots.

pH	Absorption μ moles	
	K	Mn
6.1	36.0	0.90
6.5	35.1	1.37
7.0	19.0	1.75
7.3	28.8	2.47
7.6	33.4	2.76

Table 13.

pH series with KCl in Na-phosphate buffer.
Intact seedlings.

pH	Absorption μ moles	
	Cl	K
4.0	18.9	24.8
5.0	17.7	45.1
5.8	20.2	51.6
6.9	18.8	7.0
7.7	10.3	25.9

an optimum about pH 5 to 6. It is possible that this is only a reflexion of the very marked pH-assimilation relation, and that the absorption sensu stricto is independent of the H-ion concentration above pH 4. An influence of the rate of assimilation on the absorption through promotion of growth, which is characteristic of seedlings, is indeed likely to occur

also in older plants, though of course less marked. — Table 13 indicates a decrease in Cl absorption near pH 8; this was, however, not constant, and in other series it was totally absent. As usual the conditions in the most alkaline solutions are somewhat variable, which seems to be a matter of minor importance. — In spite of the very constant anion absorption the absorption minimum for K at pH 7 is very pronounced.

Explanation of the depression at pH 7.

It is a well-known fact that the growth of plants is often depressed by a reaction of pH 7 in the nutrient medium. PIRSCHLE (1931) has brought together examples from the earlier literature and has given new evidences in his own work. He has also called attention to the probable relation of the growth minimum to the common occurrence of chlorosis at this reaction. The problem of chlorosis has been thoroughly studied by OLSEN (1935), who has also advanced a theory for explaining it.

It seems a priori conceivable that these cases of chlorosis and growth depression should be related to the established minimum of cation absorption and nitrate assimilation. Indubitably a retarded nitrate assimilation must cause chlorosis and this together with the diminished uptake of cations result in decreased growth rate. It was thus necessary to see whether the results of OLSEN were in conformity with ours.

OLSEN has come to the following conclusions. At pH 6 to 7 the plants absorb such large amounts of phosphate that iron is precipitated in insoluble form within the plant and thus rendered inactive. At higher pH, the phosphoric acid is precipitated by Ca in the nutrient medium, the absorption decreased and the iron made available.

In discussing the statement of OLSEN that the P-absorption has a maximum at pH 7 it must be remarked, that he has calculated the »absorption» as the concentrations of the elements in the plants on weight basis.

As emphasized earlier (p. 134) these concentrations are affected by the growth rate and are no real measure of the actual rate of absorption. The high P-concentrations of the tissues might as well be the consequence as the cause of retarded growth.

Nevertheless, OLSEN has shown that chlorosis can be prevented either by the addition of iron in complex compounds, which are not precipitated by phosphoric acid, or by decreasing the P-content of the nutrient medium. This could be brought about directly or indirectly by increasing the Ca-content and thereby precipitating Ca-phosphates.

A comparison of the conditions for chlorosis with the depression of cation absorption and nitrate assimilation is very instructive. Superficially the agreement is to some extent striking.

It has thus been demonstrated, that decreasing the phosphate additions both prevents chlorosis and increases the nitrate assimilation. The beneficial action of calcium was not very marked in our experiments; on the other hand a stronger improvement was obtained with magnesium. — With regard to the effect of complex iron the results are more inconsistent.

However, the explanations of OLSEN can not be applied in our case. The subsequent rise in nitrate assimilation above pH 7 is not due to a decreased solubility of phosphates as the phenomenon is more apparent in Ca free solutions where no precipitation occurs. Moreover, the effect of phosphate in our case is indubitably connected with the concentration of the cations and not so much with the phosphoric acid itself. Finally there is no reason for attributing to iron a special function in our experiments.

The chlorosis studied by OLSEN is evidently not related to the depression of nitrate assimilation. It seems as if an acidity of pH 7 should in different ways offer critical conditions to the normal nutrition of plants.

From our experimental results certain conclusions might be drawn for explaining the depression of nitrate assimilation. It has already been emphasized that the primary effect

of the reaction is a decrease in cation absorption. Because of the reduced or inhibited manganese absorption the assimilation is rendered impossible.

If this explanation be correct, assimilation of nitrate must always appear if absorption of manganese can be promoted.

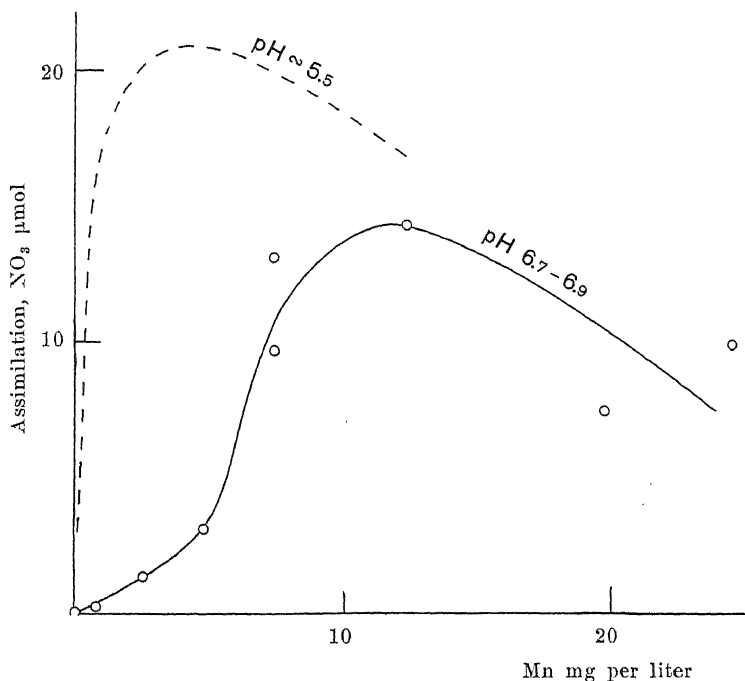


Fig. 9. The relation between manganese content of nutrient solutions and nitrate assimilation by excised roots at pH 5.5 (average curve, cf. BURSTRÖM 1939 *a*) and pH 6.7-6.9 (curve drawn free hand through the individual points).

That this is possible through a convenient balancing of the nutrient solution has already been shown. Another way is to strongly increase the Mn-concentration of the solutions.

Two series of experiments with Na-phosphate buffers at pH 7 and varied Mn-concentrations are shown graphically in fig. 9. On the same diagram the mean curve for the influence of the Mn-concentration on assimilation at pH 5.5 has been

plotted. This shows an optimum assimilation already at about 4 mg Mn per liter; at pH 7 the optimum assimilation is not obtained until the very high concentration 12 mg per liter is reached. Not even at this point can the assimilation be brought up to maximal level. It should be noted in this connection that the same assimilation as promoted by 12 mg Mn at pH 7 is obtained by 0.5 mg at pH 5.5.

It must be pointed out, however, that this effect of increasing the Mn content is quite different from the superficially similar effects of increased Mg or Ca concentrations. These are obtained only if Mn is also present. The Mn-effect, on the other hand, can be obtained as well without the additions of other cations.

From what has been said above the central position of manganese for regulating the nitrate assimilation even at pH 7 is evident. The depression of the assimilation can be fully explained on the basis of the manganese absorption and the balance of manganese to other cations.

This is also the reason why the total ion concentration had no effect in the experiments with mature plants but did exert an influence with seedlings. The former experiments were performed with solutions not purified from heavy metals and without additions of manganese. The solutions as well as the roots, which had been raised in complete nutrient solutions, contained apparently minute quantities of manganese, sufficient to promote some assimilation. The manganese content was, nevertheless, very low and even if the other salts were varied within wide limits, they were still in excess over the manganese. The depression was thus always very marked. The seedlings, on the contrary, were amply supplied with manganese. Through decreasing the buffer concentrations, further increasing the Mn content, or balancing the solutions, the manganese absorption could easily be brought up to such a level as to maintain a nearly normal rate of assimilation even at pH 7.

It might be pertinent to direct the attention to the following facts which are of interest from methodical point of view. In a solution of pH 7 containing very little manganese, the assimilation of nitrate can be promoted in either of two ways: (1) by increasing the Mn-content or (2) by adding for example Mg. Mn and Mg apparently produce the same effects but a

substitution of one element by the other is, nevertheless, out of the question. They only bring about the same end result in two different ways. The same might also be true of their effects on growth in such a case.

It is instructive to compare the action of magnesium and complete nutrient solution on manganese absorption and nitrate assimilation at pH 7. It has previously been emphasized, that a manganese absorption of about 0.3 μmol s represent the level, below which the nitrate assimilation is always diminished. This level has been marked on fig. 8. In unbalanced solution — curve A — only the absorption values between pH 6.5 and 7 fall below this level in accordance with the depression of assimilation. Addition of magnesium — curve B — increases the Mn absorption only slightly but nevertheless sufficiently to raise it above the critical level and render maximal assimilation possible. Through further addition of Ca and Fe — in complete solution, curve C — the absorption rises enormously, but the assimilation very little (cf. tables 5 and 8). This heavy increase in Mn absorption represents a luxury consumption, which can not be utilized by the roots (cf. BURSTRÖM 1939 c).

H-ion concentration and cation absorption.

The cause of the depression of the cation absorption at pH 7 can not be entirely decided at present. The laws of cation absorption and its relation to external and internal factors are — in spite of the enormous literature on mineral nutrition — very inexactly known. This is true also of the influence of H-ion concentration on the mechanism of ion absorption. In most works dealing with the effects of the reaction, the mechanism of absorption is made obscure through interaction of anions and cations with plant growth, the results thus being difficult to interpret correctly.

It is generally assumed — often rather a priori — that the absorption of cations increases with decreasing H-ion concentration; there are, however, also authors denying this conception (for example COLLANDER 1935, JACQUES 1936 b). LUNDE-

GÄRDH (1938, 1939) has recently collected data on the potentials and exchange of ions at root surfaces; his studies have provided additional evidence that the initial stage in cation absorption is an adsorption by the plasm colloids. From this point of view the rise in cation absorption in our experiments from an acid reaction to pH 6 and also the rise from pH 7 to 8, or in balanced solutions the continuous increase over the whole range of pH, are only in accordance with the rules for the adsorption of cations to a negatively charged colloid. The decrease from pH 6 to 7, on the contrary, needs an explanation.

The decrease appears only in unbalanced solutions, that is to say, in solutions containing a high content of monovalent cations compared to bivalent. It might be assumed, therefore, that the continuously increasing absorption with increasing pH represents the pure adsorption to plasm surfaces, which have a certain degree of stability because of a convenient balance of absorbed mono- and bivalent cations. The depression from pH 6 to 7 might consequently be caused by an instability of the plasm in unbalanced solutions, perhaps by an unfavourable hydration of the surface due to the exclusive or predominating adsorption of monovalent cations.

Two points in fig. 8 are noteworthy. In the first place, the manganese absorption at pH 6 is rather independent of the balancing of the solutions. Secondly, the largest difference between unbalanced and balanced solutions is found at the most alkaline reactions. LUNDEGÄRDH (1938) has previously shown that within the pH range 3 to 6 the absorption to the root surface even in unbalanced solutions smoothly follows the physico-chemical expectations in a colloid of constant composition. It seems as if the plasm surface were quite stable at $\text{pH} < 6$ even in unbalanced solutions and consequently no disturbances appear in the absorption of cations. From pH 6 towards an alkaline reaction the difference between the absorption from balanced and unbalanced solutions continually increases. Of course no exact mathematical computation can

be made on these curves, but the difference between curves A and C approximately forms a straight line when plotted against pH, beginning from zero at pH 6 and attaining maximum at alkaline reaction. This difference shows the influence of a depressing factor, which impedes the absorption from unbalanced solutions. Emphasis must be laid upon the fact that this factor continually increases from pH 6. This means that the subsequent rise in the absorption at $\text{pH} > 7$ is not due to a disappearance of the disturbances but to the fact, that the factor promoting absorption — the capacity for adsorption — increases at an accelerated rate, thus neutralizing the depressing effect. The simple shape of the curves forming the basis for the cation absorption indicates some simple physico-chemical properties of the plasm. What causes this depressing effect can not be decided at present.

The two factors affecting cation absorption namely: the promoting effect of the capacity for adsorption over the whole range of pH, and the depressing effect at $\text{pH} > 6$, together explain the wave-shaped absorption curve in unbalanced solutions, with minima at pH 3 and 7 and maxima at pH 5.5 to 6 and 8. In balanced solutions with stabilized plasm surfaces, the depressing factor will not be in evidence and the absorption will rise continually with decreasing H-ion concentration.

The retarded absorption of cations has probably a certain ecological importance. On soils with an unfavourable balance of ions it is possible that the cation absorption might be decreased and the growth impaired at neutral reaction.

It can be assumed, that some reported cases of chlorosis and growth depression at pH 7 might be connected with diminished cation absorption and nitrate assimilation, though OLSEN (1935) has shown that other factors are also involved.

Specially worth mentioning is the occurrence of manganese deficiency. This disease, known as grey-speck-disease, is common on oats which are grown on soils at a pH of 6.5—7.5 but is nearly absent on the more acid

and alkaline soils (LUNDEGÅRDH 1932). This is of course partly connected with the increased solubility of manganese at acid reaction (OLSEN 1934), but on alkaline soils this argument is not valid. LUNDEGÅRDH also found that a disturbed balance of ions, manifested by a high quotient $K:Ca$, favoured the appearance of the disease. These observations which could not be fully explained at the time, are, however, in good agreement with the recently discovered rules governing the absorption of manganese. — As to the absorption of this ion OLSEN has stated that in water cultures it reaches a maximum at pH 7 and decreases towards both acid and alkaline reaction. This is quite the reverse of our experiments with unbalanced solutions and not even in agreement with the results of complete nutrient solutions. The absorption of OLSEN denotes, however, the Mn content of the plant tissues which gives little evidence as to the actual rate of absorption.

5. Concluding remarks.

As mentioned already, the nitrate absorption is often supposed to be promoted at an acid reaction. Most publications dealing with this problem are, however, suffering from the methodical errors discussed in the introduction. Very often the duration of the experiments has been chosen so long as to render a distinction between indirect and direct actions on the nitrate absorption impossible. It is evident that the reaction exerts a very marked indirect influence upon the assimilation even in short time tests and therefore it might be difficult to ascertain, whether a certain change of nitrate utilization is primarily connected with absorption or assimilation. It must also be pointed out, that only testing the utilization at a few reactions can lead to severe misinterpretations. The whole pH range must be studied and other conditions must be varied as well as the reaction to give a complete picture of the problem.

From our results it might be possible to conclude that the nitrate absorption is not directly influenced by the reaction. In the experiments with intact plants — mature or seedlings — a slightly indicated maximum absorption at pH 5 to 6 was obtained. The deviations from the average absorption

were, however, only small and it seems very likely that the optimum is induced by the very marked maximum of assimilation at this reaction. This interpretation gained in probability as it was shown that the chlorine absorption was not in the least affected by the reaction. It must be assumed, however, that the mechanism is the same for nitrate and chlorine absorption, both being monovalent, strong anions not forming insoluble compounds within the plants. According to our knowledge, no earlier data are available concerning the rate of chlorine absorption at different reactions, though this ion seems to be the most convenient for studies on the mechanism of anion absorption. The difficulties involved in the absorption of nitrate have been emphasized in the preceding discussion. The absorption of phosphates, which has been thoroughly studied by several authors, is mainly regulated by their solubility and dissociation. The latter factor is of still greater importance when one considers the penetration of weak acids, as for example H_2S and CO_2 , which has been especially studied by OSTERHOUT and his associates (cf. JACQUES 1936 *a*). These acids are apparently diffusing through the plasm as undisassociated molecules, a process which has little bearing on the mechanism of ion intake. JACQUES (1937) has, however, also investigated the rate of absorption of iodine by *Valonia* cells, finding it independent of the reaction. This observation is in good agreement with our results on chlorine absorption.

The fact that the absorption of anions is not influenced by the reaction, whereas the cation absorption is strongly affected, is additional support for the opinion advanced by LUNDEGÅRDH & BURSTRÖM (1933) and further elaborated by LUNDEGÅRDH (1935) that the mechanisms of anion and cation absorption are quite different. According to this theory, the driving power of the absorption of anions is the anion-respiration, and there is no immediate reason for assuming a connection of this mechanism with the reaction of the external solution.

In one way our results have confirmed the opinion of CLARK & SHIVE (1934 *a, b*) that the reaction of the nutrient

medium is affecting the rate of assimilation within the roots. But it has also been shown that the reaction acts only indirectly, and that a direct relationship is not very probable. ECKERSON (1924), DITTRICH (1930), and BURSTRÖM (1939 *a*) all agree that the reduction of nitrate has its optimum at a pH of about 7.5, while the optimum reaction of the nutrient medium is found at pH 6 or lower. It has also been shown, that only the absorption of cations, or more correctly the adsorption of cations on the plasm surface, is directly influenced by the external reaction. The optimal cation absorption appears either at pH about 6 or at an alkaline reaction, owing to the compositions of the nutrient solutions, and especially to the balance of mono- and bivalent cations.

As a result of the different absorption of cations other processes in the roots are regulated. That of first importance is the growth rate, which usually shows a relation to the pH corresponding to that of the cation absorption. Independent of the growth rate the process of nitrate assimilation is also affected, because of its relation to the intake of manganese and the absorption of this ion to the plasm.

The physiological importance of the reaction consequently should be limited to a direct influence on the cation absorption, apart from the detrimental effects of very acid and alkaline reactions. But only in water cultures has the reaction such a restricted importance. In soils there are other circumstances, perhaps of greater ecological importance, as for example the regulation of solubilities, hydration and water holding capacity of soil colloids, base exchange etc.

A good illustration of the influence of the reaction on ion absorption and growth is given by PIRSCHLE (1931) who, summarizing a number of experiments with different plants, writes that "— — maximale Aufnahme von N und P_2O_5 in den meisten Fällen bei pH 7 stattfindet, also bei neutraler Reaktion, wo in der Regel ein Wachstumsoptimum nicht vorhanden ist. Die Mehrzahl der maximalen Aufnahmen von K_2O fällt auf pH 5 und damit einigermaßen mit dem häufigsten Wachstumsoptimum (pH 5—6) zusammen." — »Aufnahme« here denotes the concentrations of the elements within the mature plants on a dry weight basis. The maximum concentra-

tions of the acids are found at a minimum of growth, that is at pH 7; this means, that high concentrations are probably only the consequence of retarded growth. For K on the contrary, maximum concentration coincides with growth maximum; it might therefore be believed that the reaction determines the absorption which in its turn promotes growth.

6. Summary.

This paper describes experiments on the influence of the H-ion concentration of the nutrient medium on the absorption, accumulation, and assimilation of nitrate by wheat plants. In addition the importance of the reaction for the absorption of ions in general is discussed.

The experiments were carried out partly with old plants in flowing and stagnant solutions, and partly with aseptic seedlings or excised roots in aseptic solutions.

The methods employed are thoroughly discussed. Special stress has been laid upon the necessity of separating nitrate absorption and assimilation from each other, and the necessity of avoiding interference with the growth rate.

The results obtained may be summarized as follows:

1) The H-ion concentration does not seem to have any direct influence upon the rate of anion absorption by the roots. This is obvious with regard to chlorine. With nitrate a flat optimum of absorption was obtained about pH 5 to 6, probably due to the very pronounced influence of the reaction upon the assimilation, which gives a slight reflexion in the rate of absorption. It must be emphasized also that according to the current theory of anion absorption no relation to the external reaction must be postulated.

2) The H-ion concentration has a very marked influence on the absorption of cations (Mn and K determined). In balanced solutions as for example complete nutrient solutions, the Mn absorption rises continuously with increasing pH. This was explained by the favoured adsorption to the negatively charged

plasm surface. In unbalanced solutions, without bivalent cations, K and Mn absorption show a minimum at pH 7, and rise towards both acid and alkaline sides, reaching maxima at pH 5 to 6 and >8 . This was explained by the action of a depressing factor on cation absorption beginning at pH 6 and increasing towards alkaline reaction, thus counteracting the adsorption. Since the exact nature of this factor is unknown, it was assumed to be connected with an increasing instability of the plasm surface in unbalanced, alkaline solutions.

3) At pH 3, where irreversible disorganisation of the plasm occurs, both anion and cation absorption cease. The corresponding alkaline toxicity appears more irregular at pH 8 to 9.

4) The H-ion concentration has no direct influence on the accumulation of unreduced nitrate by the roots, beyond the case, where the roots, being supplied with abnormally high concentrations of nitrate, reach a stage of nitrate saturation. It was assumed, that such a stage might be more easily attained with unicellular plants than with roots.

5) The H-ion concentration has no direct influence on the rate of assimilation within the roots. Nevertheless, it is often of indirect importance, regulating the absorption of manganese, which, according to earlier results, is a predominating factor in nitrate assimilation. The manganese absorption is diminished or inhibited in insufficiently balanced solutions at pH 7, in which case the nitrate assimilation also shows a pronounced minimum or ceases.

The interaction of H-ion concentration and ion absorption in growth is discussed.

7. Sammanfattning.

I arbetet redogöres för inverkan av näringsmediets väteionkoncentration på veteplantors upptagning, assimilation och lagring av nitrat. I samband därmed har även berörts reaktionens betydelse för ionupptagningen i allmänhet.

Som försöksmaterial tjänade dels äldre veteplantor i rinnande eller stillastående lösningar, dels aseptiska groddplantor eller isolerade rötter av sådana i aseptiska lösningar.

De använda försöksmetoderna ha diskuterats utförligt. Särskilt har framhållits nödvändigheten av att kunna skilja nitratets upptagning och assimilation samt att kunna undvika inflytanden av plantornas tillväxthastighet på hastigheten av ionupptagning och nitratassimilation.

De viktigaste resultaten kunna sammanfattas sålunda:

1) H-ionkoncentrationen synes ej ha någon direkt inverkan på hastigheten av rötternas anionupptagning. Detta är fullt tydligt beträffande upptagningen av klor. Med nitrat erhöles en optimumkurva med ett flackt optimum för upptagningen mellan pH 5 och 6, vilket ansågs sammanhänga med den påtagliga inverkan reaktionen har på nitratets assimilation. Det framhölls även, att det enligt gängse föreställning om mekanismen vid anionupptagningen ej är nödvändigt att antaga någon direkt inverkan av reaktionen på denna.

2) H-ionkoncentrationen har en mycket stark inverkan på upptagningen av kationer (Mn och K). I balancerade lösningar, t. ex. i fullständig näringslösning, stiger Mn-upptagningen hastigt med stigande pH. Detta förklarades med en gynnad adsorption till de negativt laddade plasmaytorna. I obalancerade lösningar, utan tvåvärdiga kationer, visa K- och Mn-upptagningen minima vid pH 7 och stiga mot både sur och alkalisk reaktion med maxima vid pH 5—6 och >8. Orsaken här till ansågs ligga i en hämmande faktor, som sätter in vid pH 6 och förstärkes mot alkalisk reaktion; den motverkar således den underlättade adsorptionen. Naturen av denna faktor kunde ej fastställas, med den förmodades sammanhänga med en ökad instabilitet av plasmaytorna i obalancerade neutrala — alkaliska lösningar.

3) Vid pH 3, där irreversibla skador på plasmaytorna uppträda, upphöra både anion- och kationupptagning. Motsvarande alkalisk giftverkan visar sig mer oregelbundet vid pH 8—9.

4) H-ionkoncentrationen har ingen direkt inverkan på lagringen av oreducerat nitrat i rötterna. Undantag utgör blott det fall att rötterna utsättas för onormalt hög nitrattillförsel och synbarligen bli mättade med nitrat. Det kan antagas att ett dylikt tillstånd lättare uppnås med encelliga växter än med rötter.

5) H-ionkoncentrationen har ingen direkt inverkan på hastigheten av nitratassimilationen i rötterna. Den är dock av indirekt betydelse därför, då den reglerar upptagningen av mangan, vilken enligt tidigare erfarenheter är en dominerande faktor för nitratassimilationen. Då manganupptagningen hämmas eller helt upphör i otillräckligt avbalancerade lösningar med pH omkring 7, blir även nitratassimilationen nedsatt eller avstannar helt.

Sambandet mellan H-ionkoncentration, ionupptagning och tillväxt har diskuterats.

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The pedography of hydrologic podzol series: II. The loss on ignition and the reaction of the Annerstad series.

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The present paper is a first report on the study of a new hydrologic soil series, the Annerstad series, and is limited to the same analytical methods as were explained in the introductory work on the Unden series (MATTSON & LÖNNEMARK). The Annerstad series is the series there mentioned as being located near the south end of the lake Bolmen in the province of Småland in southern Sweden.

Description of the Annerstad series.

The profile series was dug on the northeast slope of a low sandy elevation in the midst of the Annerstad moor. The maximum elevation of this »island» of sand is only about 1 meter above the surface of the moor and measures about 100 meters in circumference.

Location: Latitude = $56^{\circ} 45' 30''$, east longitude = $13^{\circ} 44' 06''$.

The trench was dug a few meters north of the highway which runs from east to west (Cf. fig. 12 b. The boy stands on the highest elevation just in front of the trench.)

Geology: The nearest rock outcrop, of which there is none within 1 km., consists mostly of gneiss but diorite occurs also.

The elevation of the moor is 462 feet. The surrounding country consists of low hills of gravelly material.

Climate: The data for the nearest meteorological station Lidhult, about 18 km. to the west-north-west are as follows: Average annual temperature = 5.5° C (for frost-free period = 6.0° C), rainfall 886 mm., humidity factors: Langs = 99, Martonnes = 41.

Vegetation: This might be divided into five zones (Cf. figures) as follows:

Zone I: Moor vegetation. The aerial parts of the following species were collected: *Calluna vulgaris*, *Cladonia* spp., *Empetrum nigrum*, *Erica tetralix*, *Eriophorum vaginatum*, *Hylocomium* spp., *Oxycoccus quadripetalus*, *Sphagnum acutifolium*, *Vaccinium vitis idaea*.

Zone II: The zone of small trees (birch and pine) limited to the base of the slope. Leaves of *Betula alba* and needles of *Pinus silvestris* were collected.

Zone III: Dominated by a dense cover of *Vaccinium uliginosum* of which the twigs and leaves were collected. (Cf. fig. 12 a, right side of the boy.)

Zone IV: An almost pure stand of *Molinia caerulea* (Cf. fig. 12 a, left side of the boy).

Zone V: This comprises the uppermost, level part of the elevation and consists of a dwarfed cover of different species of *Cladonia* and of *Vaccinium vitis idaea* studded with solitary groups of *Calluna vulgaris* and *Empetrum nigrum* and a few dwarfed (about 1 meter tall) *Populus tremula*. The leaves of the latter and the aerial parts of the other were collected.

We shall refer to the vegetation samples as the V-samples.

The F-layer: An F-sample was collected in each zone and consisted of the following materials (latin numerals refer to the zone):

F_I: Förra from *Scirpus caespitosus*. (No other litter available.)

F_{II}: Leaves, pine needles and twigs.

F_{III}: The same as F_{II} with some remains of *Molinia caerulea*.

F_{IV}: Förna from *Molinia caerulea*.

F_V: Lichens, leaves and twigs.

The profile series: On July 25 1939 a six meters long trench was dug from the top of the slope and extending about two meters out into the level moor. The ground water seeped in at the wet end at a depth of 46 cm.

The profile series, which is partly shown in the photograph in fig. 12 a, showed the following main morphological characteristics:

A₀: This horizon increas from 10 cm. at the dry end ($x=600$) to 140 cm. at the wet end ($x=0$. Cf. fig. 13 a).

A₂: This horizon forms a bleached layer which extends through the entire series varying in thickness up to 15 cm. Toward the wet end the colour becomes darker through a higher humus content but the presence of an A₂ horizon is unmistakeable even under the thickest layer of peat at the wet end. How far into the moor this horizon has developed we do not know since we could only with difficulty penetrate as far as we did.

B: The B horizons change, at the dry end, from dark brown to light brown and light gray and, at the wet end, from brownish gray through brown to yellowish brown.

C: The parent material consists of fine sand. (Cf. table 2.)

G: The gley horizon is absent. This is mentioned as a significant fact in connection with the unusual extension of the A₂ horizon.

After errecting the x and y axes as in the case of the Unden series, (Cf. figures), 129 samples were collected from 12 separate profiles as previously described. The samples were shipped to the laboratory at once, dried in the air and sifted through a 0.6 mm. sieve.

Mechanical analysis.

The mineral samples of profiles $x=0$ and $x=600$ were subjected to a mechanical analysis (table 2). The series apparently consists of quite a uniform material in which the fine

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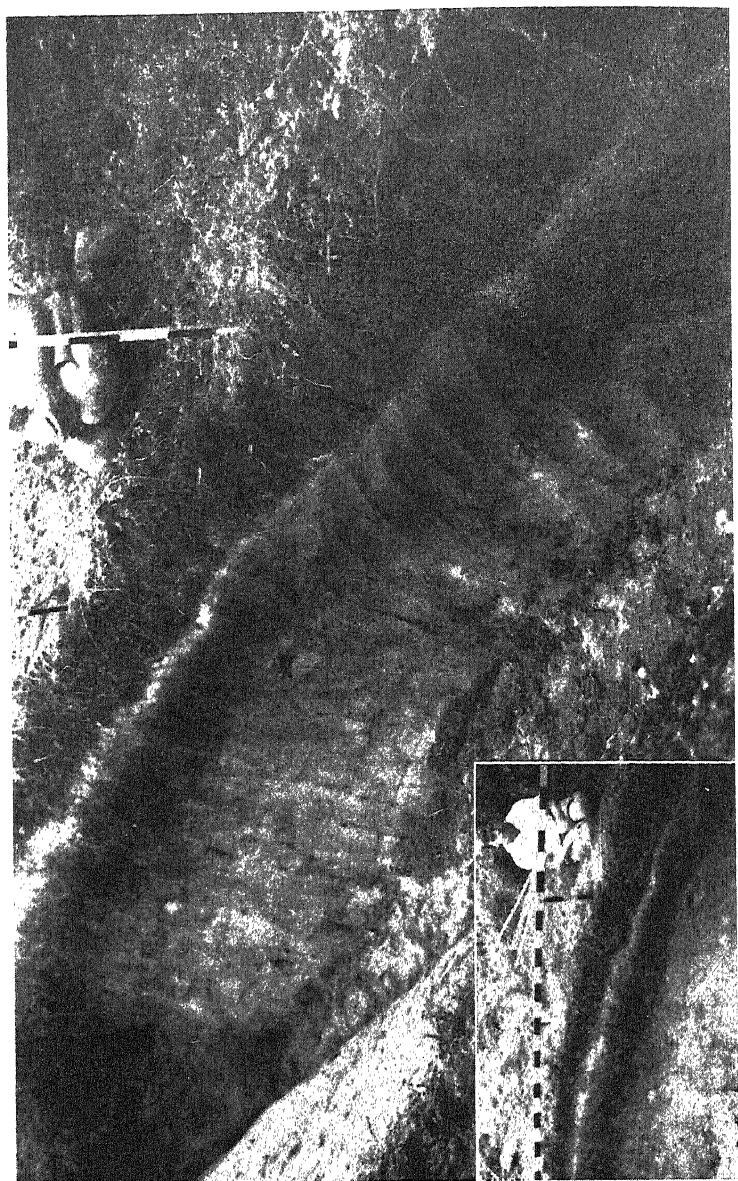


Fig. 12 a. The Annerstad series showing the upper and central parts of the profile series.

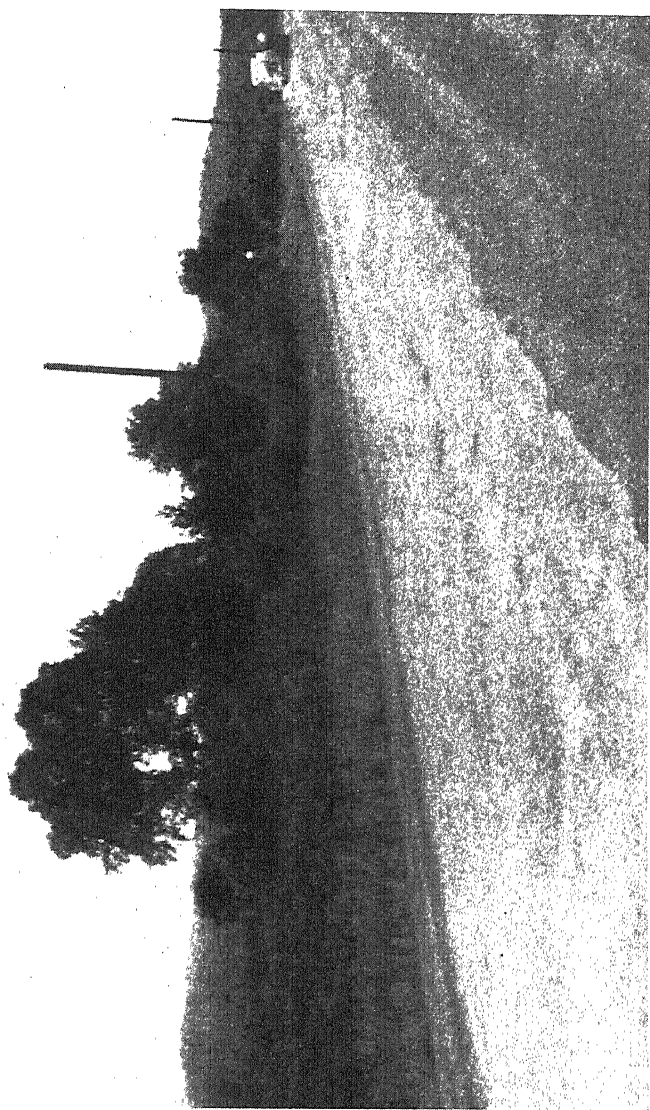


Fig. 12 h. The site of the Ammerstad series showing the »island» of sand, a part of the moor and the surrounding hills.

sand greatly dominates. The clay is more evenly distributed than in the Uden series. There is, however, an enrichment of clay in the B-horizon.

Tabbe 2.

The mechanical analysis of the Annerstad profiles $x=0$ and $x=600$. Per cent of air dry soil.

<i>y</i>	Medium ¹ sand 0.6—0.2 mm. %	Fine sand 0.2—0.02 mm. %	Silt 0.02—0.002 mm. %	Clay < 0.002 mm. %
Profile $x=0$.				
230	2.9	90.0	4.3	3.4
240	2.2	83.4	3.0	5.1
260	2.2	87.0	2.4	4.4
Profile $x=600$.				
15	1.9	85.1	—	5.0
20	1.9	91.0	4.5	3.4
25	2.1	87.7	3.2	4.6
30	1.8	82.4	2.8	6.4
40	1.6	85.9	3.4	5.1
50	2.4	89.5	2.6	4.2
60	4.6	90.9	1.5	3.1
70	2.9	91.5	2.9	3.1
80	2.5	92.0	3.2	3.2
100	3.9	92.5	1.2	3.2
120	6.4	90.9	1.5	2.8
140	5.2	89.3	4.0	3.1

Loss on ignition.

The loss on ignition of the V- and F-samples is shown in table 3 and that of the profile series in fig. 13 a and 13 b.

The unusually low values for the loss on ignition of some of the plant materials and F-samples lead us to suspect an

¹ Fraction > 0.6 mm. absent.

appreciable accumulation of dust from the highway, especially in the case of such structures which will favor the retention of the dust (i. e., mosses, lichens and litter). It is interesting to note, however, that the three species *Calluna vulgaris*, *Empetrum nigrum* and *Vaccinium vitis idaea*, which were collected at both the dry and at the wet end, show from 1 to 2 % lower loss on ignition when grown on the hill than when grown on the moor.

The loss on ignition in the profile series has two maxima both of which extend through the entire series, one in the A_0 and one in the B-horizon. This series is, therefore, with respect to the distribution of the organic matter much simpler than the Uden series.

There is a relatively large amount of organic matter in the A_2 -horizon where it falls below 2 % in only two of the samples (= 1.81 and 1.90 % in $x = 400$ and 450 respectively). The percentage is, however, not markedly higher in the wet than in the dry end.

The accumulation of organic matter in the B-horizon is considerable (from 6—15 %) but here again it is fairly evenly distributed throughout the series.

The occurrence of an eluviated (A_2) and an illuviated (B) horizon at such depths and below the ground water level must be ascribed to a special set of conditions. TAMM (1931) found no podzolization when the peat layer exceeded a thickness of 30 cm. A greater thickness is, according to him, always associated with a water table which constantly reaches up into the peat. With no downward movement of water there can be no eluviation and no illuviation; no A_2 - and no B-horizon developed. Instead we get the unstratified bluish gray (due to Fe reduction) bog soil with no accumulation of humus in the mineral horizon.

In the Annerstad series the water table reaches way up into the peat layer, at the wet end, during the driest part of the season. In the wet end of the series the podzolized horizon must, therefore, be permanently submerged. Hence submerg-

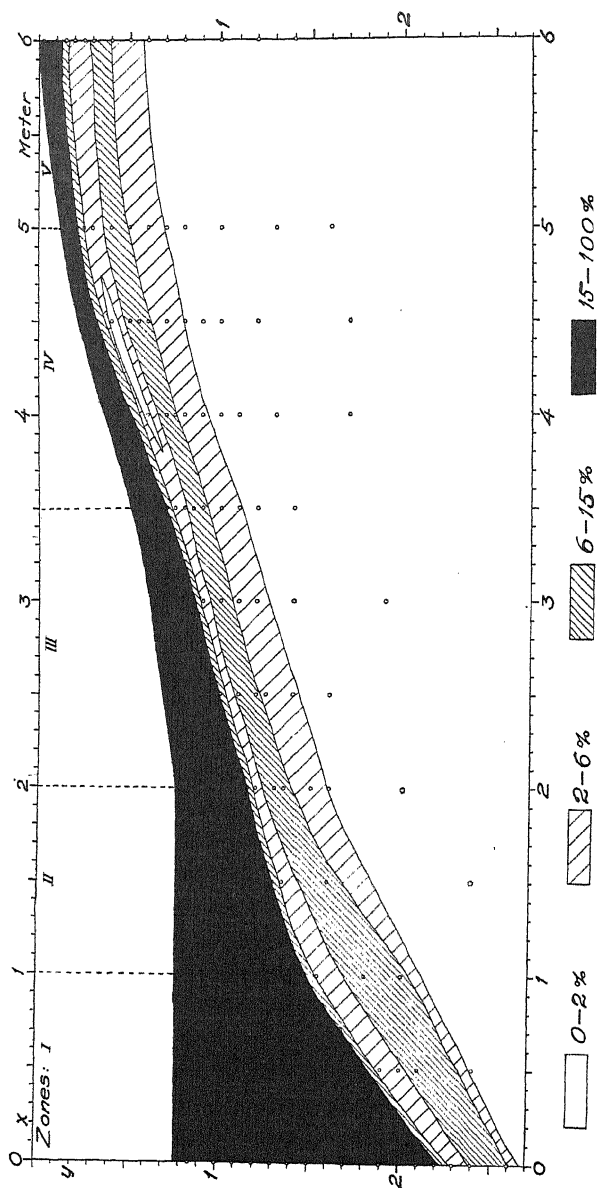


Fig. 13 a. The loss on ignition of the Annerstad series.

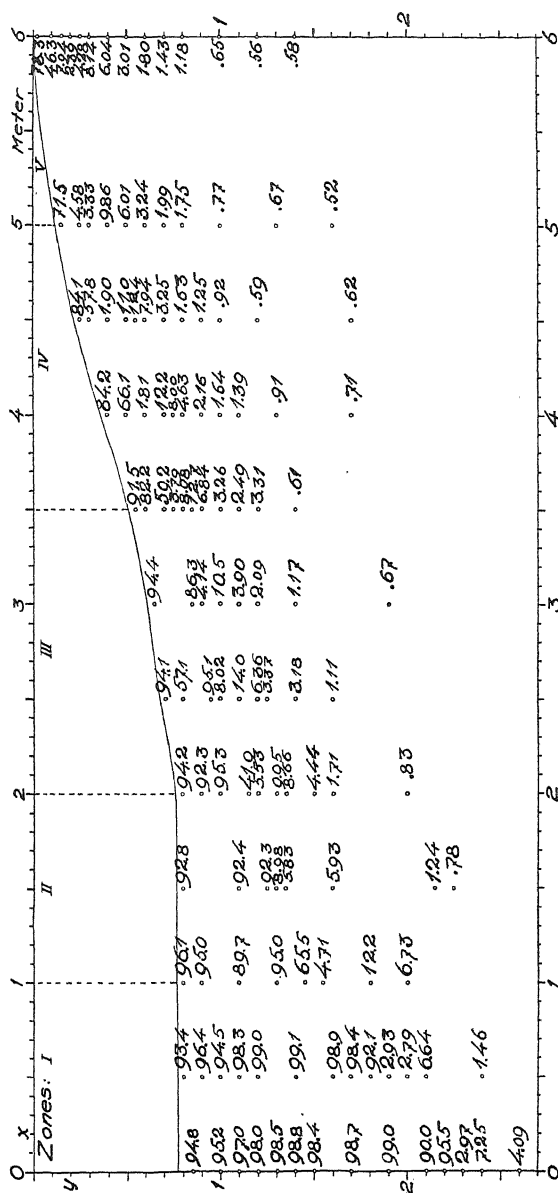


Fig. 13 b. Same as fig. 13 a.

Table 3.

The percentage loss on ignition and the pH in water, in N/100 Na₂SO₄ and in N/100 BaCl₂ of the green plant materials and of the litter (F-layer) of the Annerstad series.

Plant species	Loss on ignition %	pH in:			Difference	
		water w	N/100 Na ₂ SO ₄ s'	N/100 BaCl ₂ s''	w-s'	w-s''
Plant materials:						
Zone I:						
Calluna vulgaris	96.1	4.77	4.68	4.65	.09	.12
Cladonia spp.	91.7	4.30	4.13	3.92	.17	.32
Empetrum nigrum	95.7	4.58	4.56	4.51	.02	.07
Erica tetralix	96.8	5.09	4.99	4.92	.10	.17
Eriophorum vaginatum	96.8	5.84	5.63	5.32	.21	.52
Hylocomium spp.	80.1	4.73	4.61	4.29	.12	.44
Oxycoccus quadripetalus	96.5	4.49	4.58	4.39	-.09	.10
Sphagnum acutifolium	82.3	4.46	4.21	3.73	.25	.73
Vaccinium vitis idaea	96.1	4.54	4.60	4.54	-.06	.00
Zone II:						
Betula alba	94.9	5.08	5.16	4.97	.08	.11
Pinus silvestris	97.5	4.35	4.34	4.23	.01	.12
Zone III:						
Vaccinium uliginosum	96.7	4.30	4.34	4.27	-.04	.03
Zone IV:						
Molinia caerulea	93.7	5.35	5.35	5.28	.00	.07
Zone V:						
Calluna vulgaris	95.0	4.65	4.65	4.49	.00	.16
Empetrum nigrum	93.8	4.56	4.56	4.51	.00	.05
Populus tremula	94.8	5.95	5.68	5.63	.27	.32
Vaccinium vitis idaea	95.9	4.58	4.60	5.54	-.02	.04
F-layer.						
Zone I	95.3	4.15	3.96	3.72	.19	.43
Zone II	73.6	4.97	4.92	4.67	.05	.30
Zone III	77.7	4.91	4.85	4.54	.06	.44
Zone IV	88.9	4.39	4.34	4.04	.05	.35
Zone V	74.0	4.34	4.23	4.01	.11	.33

ence does, evidently, not preclude podzolization. This process must be the result of a downward movement of the soil solution under conditions of a negative base status (adsorbed acids in excess of adsorbed bases) independent of the ground water table.

The vertical movement of water in a submerged soil can be upward as well as downward independent of the humidity of the region.

The movement must be upward (from the mineral soil into the peat) when there is a seepage from the surrounding elevation. This might be the most common condition. This upward movement of water in the submerged mineral horizon leads to the formation of the bluish gray bog soil: Reducing conditions, relatively high base status, no infiltration (illuviation) of humus, no podzolization and no differentiation into horizons.

A downward movement of the water (from the peat into the mineral horizon) must result when the geological and topographical conditions are such that drainage is established through the more pervious mineral deposit. Ordinarily, ground water has a relatively high base status and opposes rather than induces podzolization. But if the ground water has never been in contact with a mineral deposit but represents the drainage from a sour highmoor peat its passage through the mineral soil must, even under conditions of submergence, lead to the development of a humus podzol. Such are, apparently, the conditions in the Annerstad series. The water moves from the peat into the more pervious sand through which drainage is, in some way, established. It is hoped that a study of the difference in ground water level between the dry and the wet end of the series, which is now being conducted, will solve this problem.

The pH.

As in the case of the Uden series we have determined the pH in water and in two salt solutions but instead of N/1 KCl

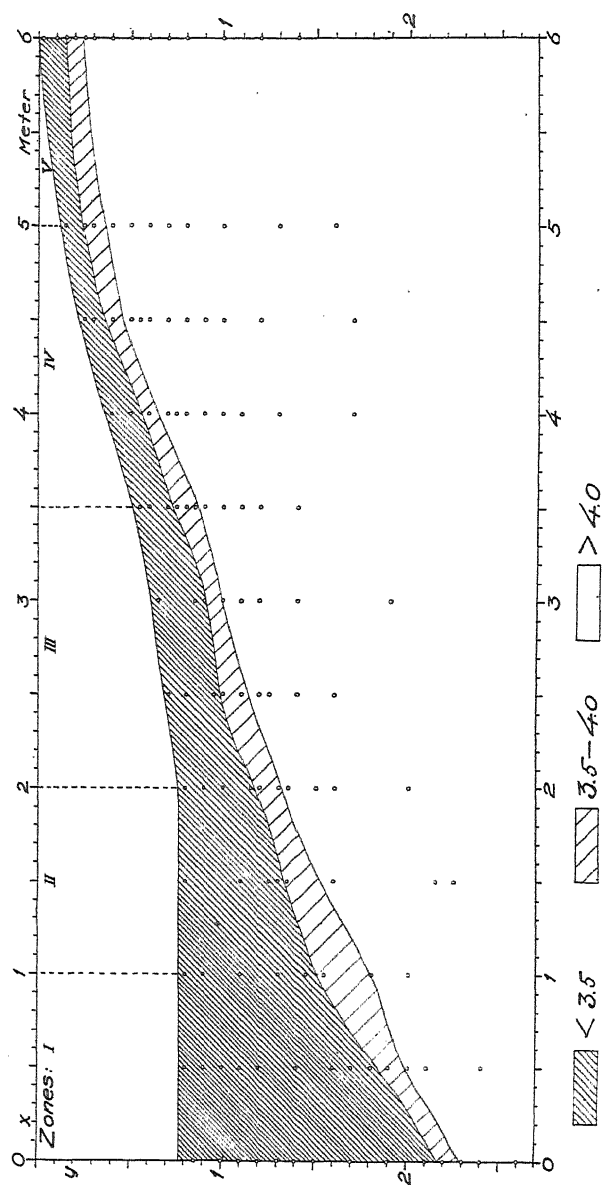


Fig. 14 a. The pH of the Annerstad series in water.

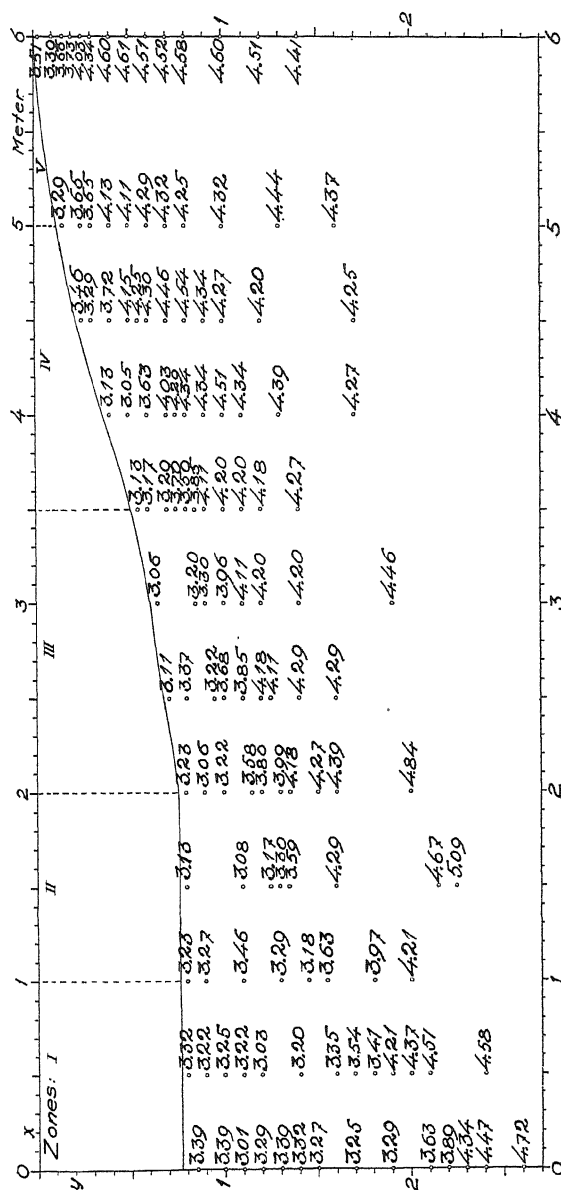
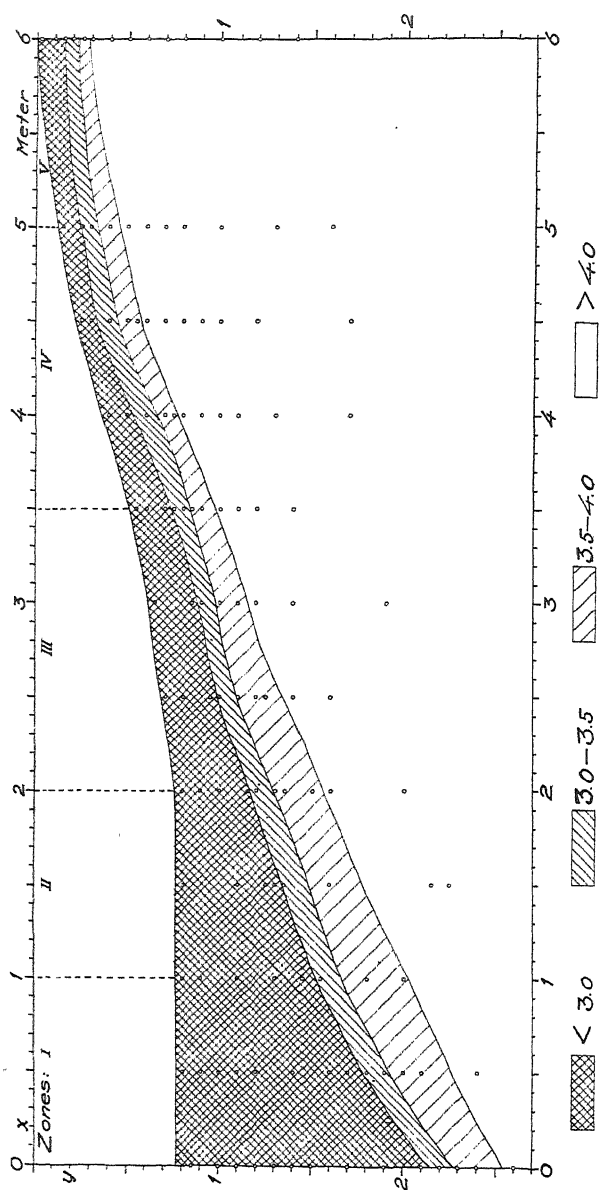
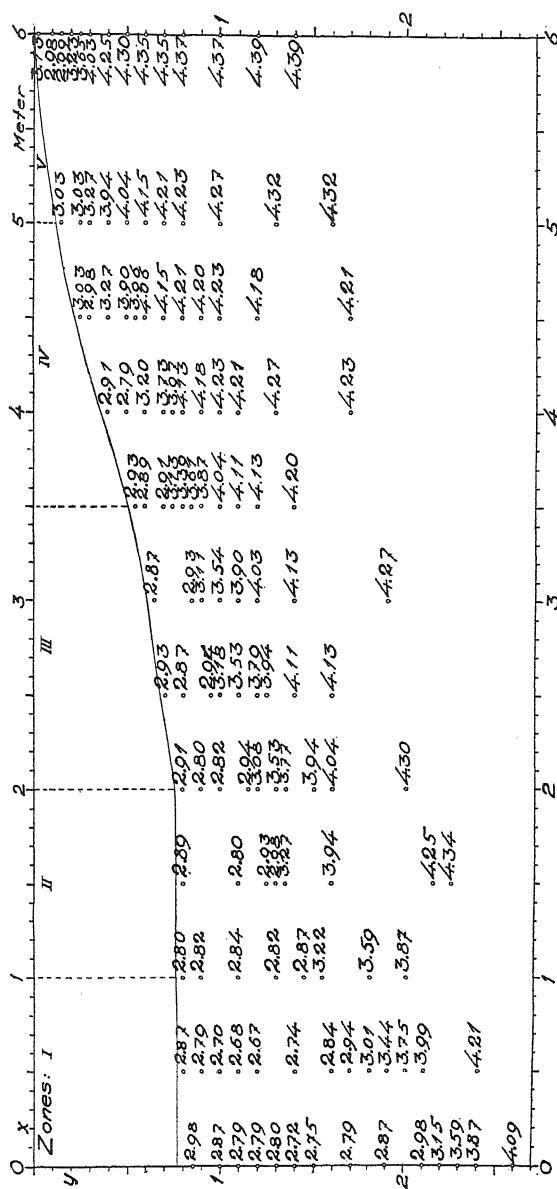
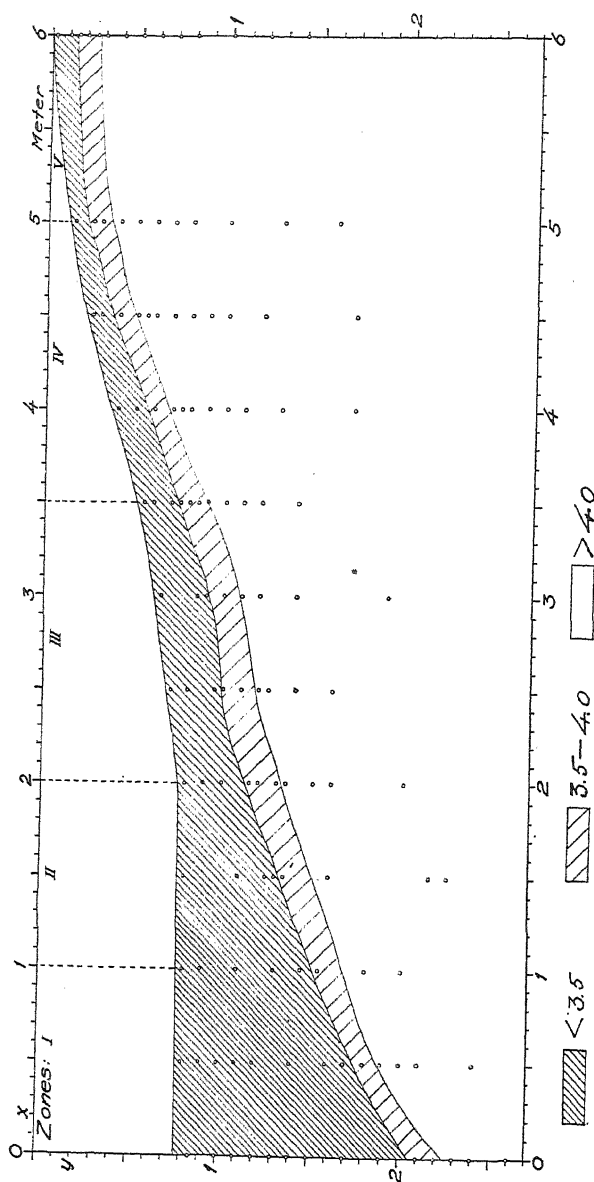


Fig. 14 b. Same as fig. 14 a.

Fig. 15 a. The pH of the Annerstad series in N/100 BaCl₂.



Fig. 16 a. The pH of the Annerstad series in N/100 Na_2SO_4 .

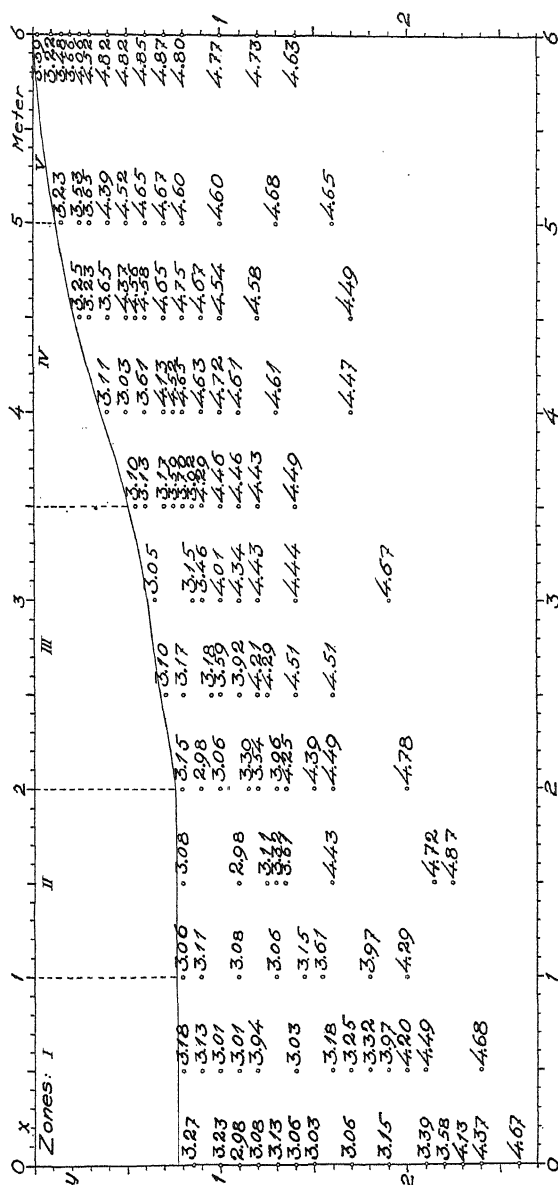


Fig. 16 b. Same as fig. 16 a.

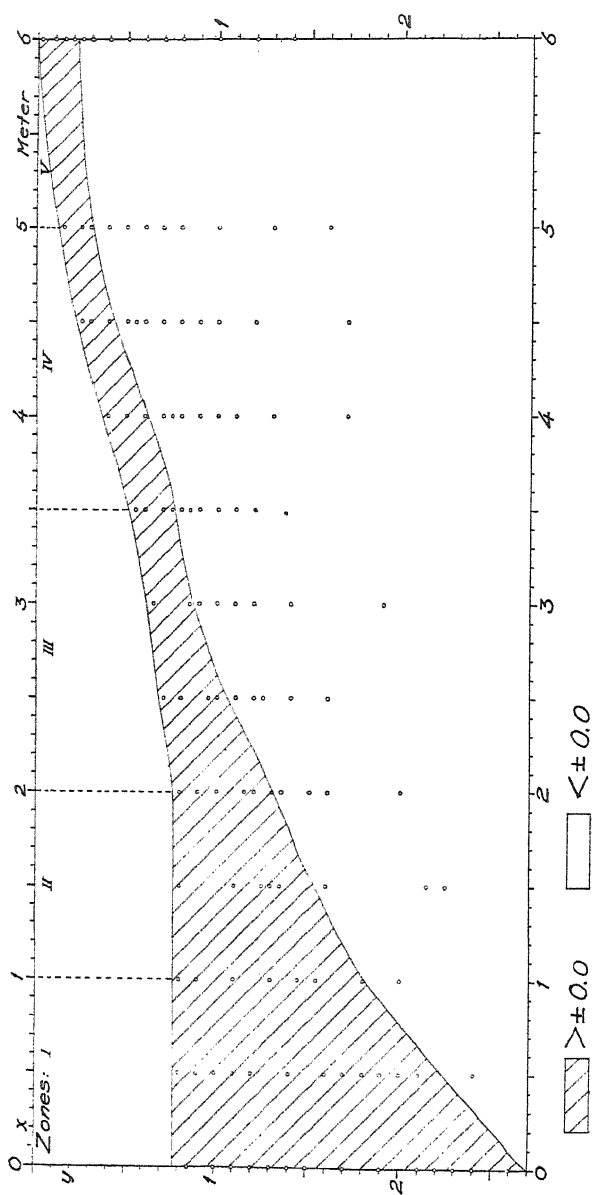


Fig. 17 a. The pH in water minus the pH in N/100 Na_2SO_4 of the Amnerstad series.

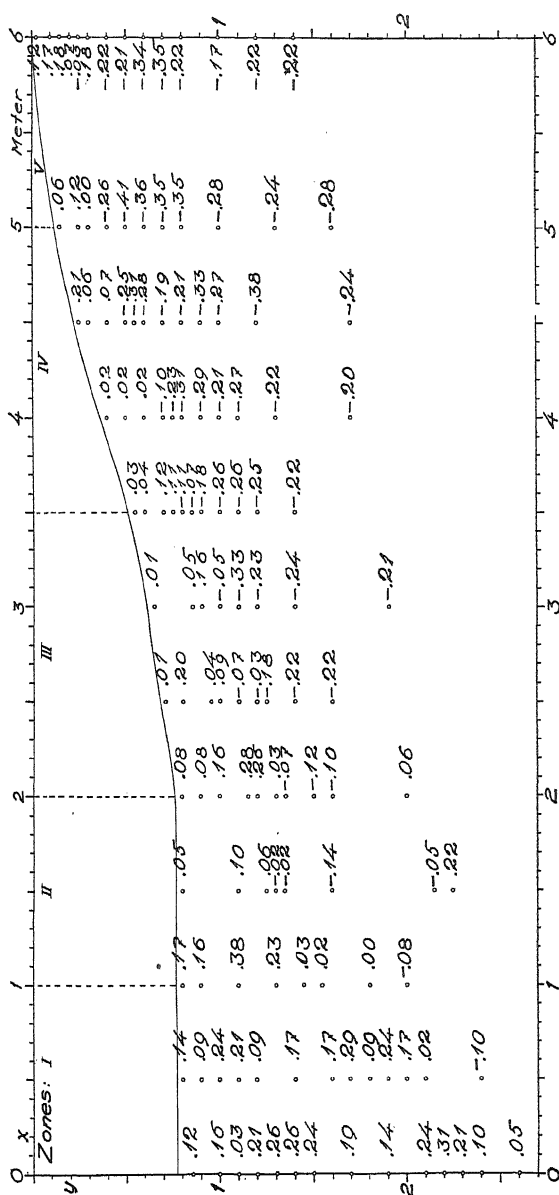


Fig. 17 b. Same as fig. 17 a.

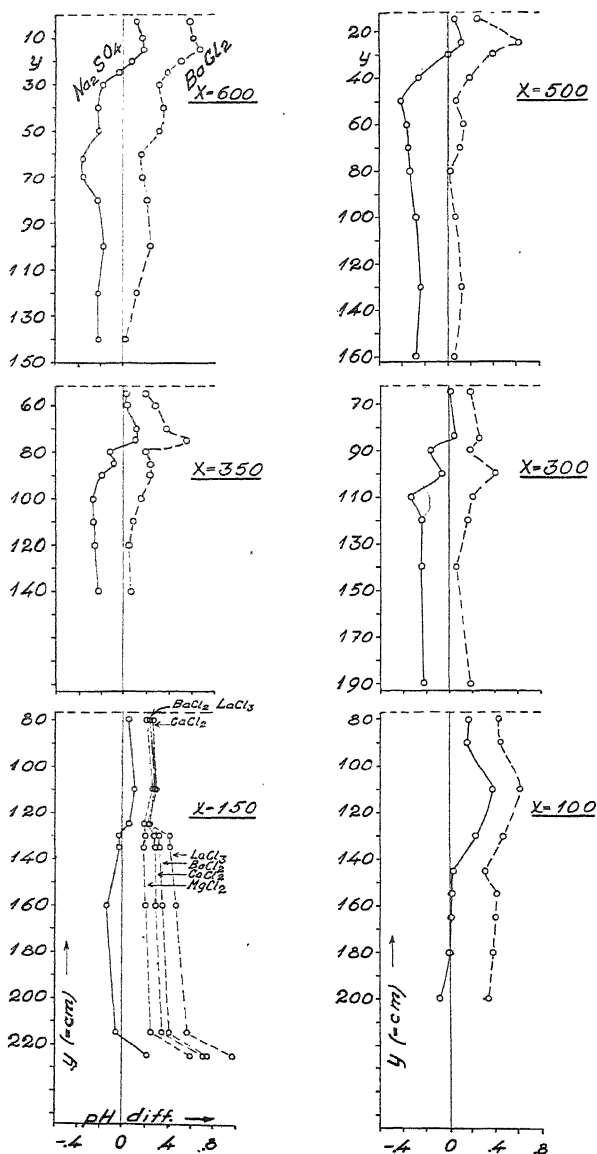
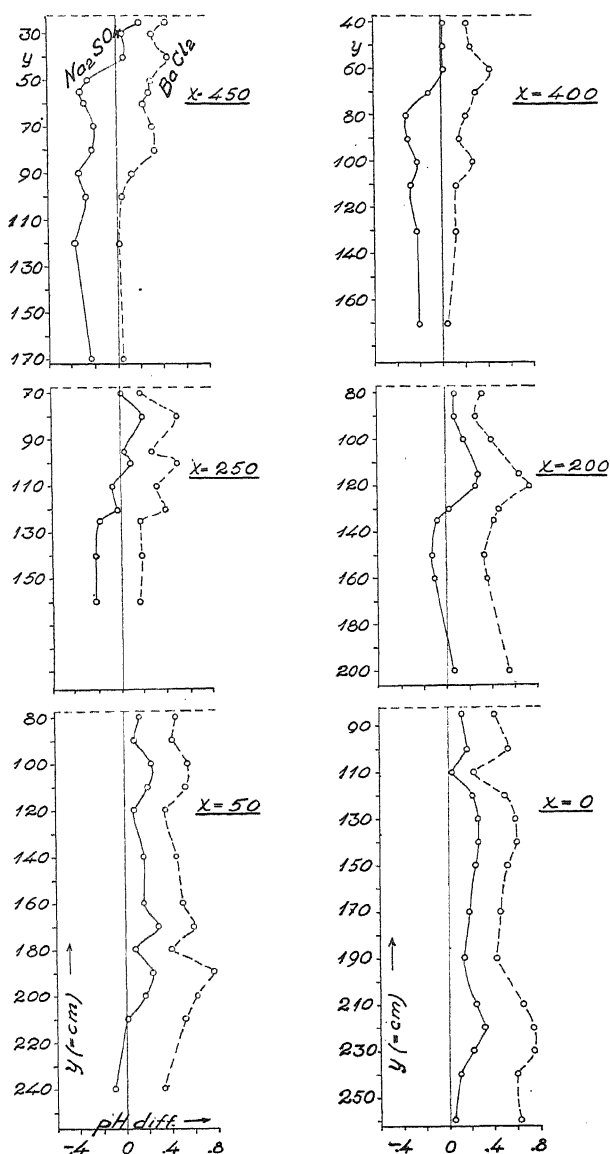


Fig. 18. The difference between the pH of the soil in water and in the in the Annerstad series of profiles. Positive values = exchange



salt solutions (N/100 Na_2SO_4 and BaCl_2) at the various depths (y-values) acidity. Negative values = exchange alkalinity.

we are now using N/100 BaCl_2 which better corresponds to the use of N/100 Na_2SO_4 in a study of amphoteric interactions.

The pH in water: The pH of the V-samples varies between 4.30 (*Cladonia* spp. and *Vaccinium uliginosum*) and 5.95 (*Populus tremula*). The pH is in every case lower in the Annerstad than in the Unden samples of the same species (table 3).

The values for the F-samples vary between 4.15 and 5.09. The high proportion of birch leaves in zones II and III undoubtedly accounts for the relatively high pH-values.

The pH in the profile series gives, like the loss on ignition, a very simple picture (fig. 14 a, 14 b). In the peat the pH is < 3.5 , in the A-horizon it is 3.5—4.0 and in the B-horizon it is > 4.0 in the entire series. The pH increases with the depth, in one profile ($x = 150$) to 5.09, but there are only a few values greater than 4.60. The lowest pH, is 3.01 (in profile $x = 0$).

The pH in N/100 BaCl_2 : The V- and the F-samples yield in most cases an appreciable lower pH in this solution than in water. In the case of *Sphagnum* moss the exchange acidity amounts to 0.73 pH-units (table 3).

In the profile series the pH of the peat has fallen below 3.0 (lowest value 2.67), the pH of most of the A-horizon has fallen below 3.50 and that of the upper B-horizon has fallen below 4.0 (fig. 15 a and 15 b). This solution yields, therefore, an exchange acidity in every sample.

The pH in N/100 Na_2SO_4 : The V- and the F-samples yield a pH in this solution which, in most of the samples, is somewhat lower than the pH in water. In several of the samples the difference is however insignificant (table 3).

In the profile series the pH values in the sulphate solution is lower in the peat and higher in the mineral horizons than the pH in water. In the peat we get exchange acidity and in the mineral horizons exchange alkalinity (fig. 16 a, 16 b, 17 a and 17 b).

Amphoteric reactions in the separate profiles.

Figure 18 shows the exchange reactions in the two salt solutions of the materials from the individual profiles.

As in the case of the Unden series the picture is a complex one and no attempt to interpret the curves will be made before the samples have been analysed. In studying the figure the following facts must be born in mind: The exchange reaction of a soil material in a given salt solution (as compared to the reaction in water) is determined by (MATTSON & WIKLANDER):

1. the concentration of the salt solution;
2. the saturation of the soil with acid or base;
3. the activity ratio of acidoids to basoids and
4. the concentration of the colloid.

As an example let us consider the fluctuations in the exchange alkalinity in the N/100 Na_2SO_4 solution.

Differences in exchange alkalinity may be due to differences in base saturation: A soil saturated with a certain amount of base does not exchange its OH ions for the anions of the salt and does, therefore, not yield any exchange alkalinity. Differences in exchange alkalinity may also be due to differences in the acidoid/basoid ratios as it in the podzol profile generally is the case. But since the maximum in exchange alkalinity occurs at a lower concentration of Na_2SO_4 the lower the proportion of basoids to acidoids, the fluctuations in exchange alkalinity will be greater when determined by the use of one single concentration of salt (i. e. N/100) instead of a concentration which produces a maximum in exchange alkalinity in each sample. Thus, where the proportion of basoids is very low, a N/100 solution of Na_2SO_4 may produce an exchange acidity whereas a more dilute solution will yield an exchange alkalinity. Differences in exchange alkalinity may, finally, be due to differences in colloid content, but here again it must be born in mind that the maximum in exchange alkalinity

occurs at a lower concentration of Na_2SO_4 the lower the colloid content of the soil.

From what has been said it is clear that the results obtained in a study of the exchange reactions in a soil profile are strictly comparable only if the samples are all as good as completely unsaturated and if the pH determinations are made in that concentration of the salt solutions which produces a maximum in valence effect as expressed by an exchange alkalinity or exchange acidity. This would, however, require a much greater number of pH determinations (in order to find the maximum effect) and has therefore not been done in the present work.

Besides the fact that it is only the mineral samples which yield an exchange alkalinity in N/100 Na_2SO_4 solution we note in fig. 18 that the difference between the pH-values in the two salt solutions (the distance between their curves) is smallest in the peat. This is probably in part due to a partial base saturation of the peat and to its low basoid content leading to an exchange acidity in both salt solutions and hence to a smaller difference.

The pH of the samples of profile $x = 150$ was determined in N/100 solutions of MgCl_2 , CaCl_2 and LaCl_3 in addition to BaCl_2 . We note a considerable increase in exchange acidity in the following order

$$\text{Mg} < \text{Ca} < \text{Ba} < \text{La}$$

but only in the mineral samples; in the peat samples there was no definite effect. The sudden increase in exchange acidity of the lowest sample ($y = 225$) in this profile in all of the salt solutions must, in view of the exceptionally high pH in water (5.09), be ascribed to a higher base status.

Summary.

The Annerstad hydrolytic podzol series covers a six meter long trench dug from a low hill into the surrounding moor. The series consists of 12 profiles from which 151 samples

were taken including 17 species of plants and 5 samples of litter.

This paper is a report on the loss on ignition and the pH in water and in N/100 Na_2SO_4 and BaCl_2 of the samples.

The Annerstad series differs from the previously reported Uden series in that it is podzolized and possesses a bleached, A_2 horizon deep under the water table and below a 140 cm layer of peat.

Sammanfattning.

Den här beskrivna hydrologiska podsolserien härstammar från en 6 meter lång grop, som grävts i en låg sandkulle i Annerstads mossen (fig. 12).

Serien utgöres av 12 profiler omfattande 151 prov, av vilka 17 utgjorde prov av växtligheten och 5 av förnan.

Avhandlingen innehåller uppgift över mekanisk analys (tabell 2), glödförlust samt pH i vatten och i N/100 Na_2SO_4 och BaCl_2 (tabell 3 samt fig. 13—18).

Annerstad-serien skiljer sig från den tidigare undersökta Uden-serien i huvudsak däruti, att den visar tydliga tecken till podsolering med blekjord djupt under grundvattnet och torven. Detta förklaras tills vidare så, att sanden genomslås av det från mossen kommande sura vattnet.

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The Metabolic Behaviour of Phosphorus. III. Balance Sheets of Phosphorus in Full-grown Rats Fed with Tri-stearine and Sodium Inositol Hexaphosphate.

By A. WESTERLUND.

From the Physiological Institute.

The present paper is the third in a series on the metabolic behaviour of phosphorus and is concerned with inositol phosphorus. This form of phosphorus has a wide distribution in food stuffs, especially the cereals. McCANCE and WIDDOWSON (1935) have analyzed the edible grains from a number of cultivated plants and have found the inositol phosphorus to vary from 41 to 68 per cent of the total phosphorus present. Investigations of the variation of these percentages within one and the same species have been published by HARRIS and BUNKER (1934) for maize only. Half of their determinations lie between 65 and 74 per cent of the total content.

Different opinions as to the amount of phosphorus which can be absorbed by the intestines from the inositol compound have been set forth in the literature. Generally, a hydrolysis is assumed to be the initial phase. But whether the requisite phosphatase is excreted actively by the intestinal mucosa or passively brought to the intestines in the cereal part of the diet, does not seem to have been settled. Possibly there are dissimilarities between different species in this respect.

In a long series of investigations MELLANBY (1937) has shown that cereal grains, especially oats, must be considered directly rhachitidogenic for dogs and rats. The responsible factor does

not seem to be any of the three dietetic characteristics which the classical concept of the rhachitidogenesis regards as sufficient, viz. (1) a low supply of vitamin D, (2) a low supply of total phosphorus, (3) an abnormal proportion of calcium to total phosphorus in the food. In spite of many attempts, MELLANBY failed to get any idea of the nature of the rickets producing factor.

The question was taken up by BRUCE and CALLOW (1934), who found that sodium inositol hexaphosphate does not prevent common rat rickets, when added to a diet low in vitamin D and phosphorus, and high in calcium. They conclude that inositol phosphorus is less available for intestinal absorption than inorganic phosphorus, and see in this fact one of the reasons for MELLANBY's result.

HARRIS and BUNKER (1934) discussed the problem from another point of view. They sought for a positive covariation between the dietary content of inositol phosphorus and the severity of rickets, given by them as a number of plus up to four. Their results have been summarized by the present writer as follows:

Degree of rickets	Mean of the percentage of inositol-P in the diet	Number of animals
0	.183	6
1	.172	7
2	.177	13
3	.181	13
4	.202	1

If the rickets resisting group of animals is omitted, the positive covariation between the inositol phosphorus content in the diet and the degree of rhachitis is quite obvious. But this covariation is far from being significant, as the differences between the means of inositol phosphorus content are not significant. The entire set of inositol phosphorus determinations may be considered homogeneous. HARRIS and BUNKER warn not to lay too much emphasis on the content of inositol

phosphorus in rachitidogenic diets rich in cereals, and suggest the existence of other factors which may possess a much heavier effect than sodium inositol hexaphosphate.

Direct determinations of the intestinal availability of inositol phosphorus in human beings have been performed by McCANCE and WIDDOWSON (1935). They have fed large amounts of phosphorus as calcium magnesium inositol hexaphosphate, with the patented name »phytin», and have used the traditional method of determining the difference between the oral intake and the fecal outgo. No record of the intake of calcium is given. Of the four experimental subjects, the three adults (one man and two women) eliminated in an unchanged form an average of 46 per cent of the inositol phosphorus ingested. The child, a boy of four years, eliminated 23 per cent, but this experiment is said not to have been so reliable as the others. This investigation shows that a certain, though variable, amount of the inositol phosphorus ingested may be considered unavailable for intestinal absorption, but it does not give any opinion as to the fate of the remaining part (54 and 77 per cent respectively). A considerable part of this may have been hydrolysed in a caudal niveau of the bowel, the phosphorus absorbing capacity of which however is poor. As a rule, the content of total phosphorus in the intestinal excreta was considerably greater than the quantity of inositol phosphorus ingested.

On the basis of their chemical analyses of different food materials, McCANCE and WIDDOWSON (1935) tried to evaluate the consumption of inositol phosphorus from freely chosen diets in 63 men and 63 women of the English middle class. They found that a maximum value of about 20 per cent of the phosphorus intake is in the form of inositol phosphorus. This fact holds good only for England, where the middle class derives most of the phosphorus consumed from animal and not from vegetable sources. With the poorer classes, especially in other countries, where cereals constitute a more substantial part of the diet, the consumption of *total* phosphorus may

point in the wrong direction as to the estimation of the intake of *available* phosphorus.

LOWE and STEENBOCK (1936), using adult rats (femals) as test objects, have confirmed the observations of McCANCE and WIDDOWSON as to the low availability of inositol phosphorus. Furthermore, they have found that the consumption of calcium must be considered, as an addition of CaCO_3 is followed by a marked decrease in the intestinal availability of the inositol phosphorus. This depression is demonstrated by an enormous drop in the renal excretion of phosphorus, and by the fact that the total excretion of *inorganic* phosphorus is reduced to about one fifth. The authors conclude that the intestinal flora must be given much consideration in the solution of the problem of the intestinal availability of inositol phosphorus.

The experiments hitherto reviewed have been performed using intact animals as test objects. Attempts, by vivisectionary methods, to determine directly the intestinal absorption of phosphorus from different sources have been made by LASKOWSKY (1937) in Verzár's laboratory at Bâle. He injected 3 ccm of the phosphate solutions used into two strangulated loops of the small intestines in adult rats after laparotomy, and closed the abdominal wall. After one hour the abdomen was reopened, and the phosphorus content in the loops was determined. The results showed a small difference in the intestinal capacity to absorb sodium phosphate and sodium inositol hexaphosphate, but the difference was quite insignificant, as is shown by the following analysis:

$$\text{difference} = .0651 \text{ per unum } (= 6.51 \text{ per cent}),$$

$$s^2(1/9 + 1/5) = .007410,$$

$$t = .76; \quad n = 12.$$

The corresponding value of P (probability) in FISHER's (1930) Table IV is in the region of .5, and cannot be regarded as significant.

On the other hand, LASKOWSKY's experiments demonstrated a significant difference in the phosphorus absorbing power between the cranial and the caudal loop, the former being more effective than the latter. — The conclusion to be drawn from the experiments now under consideration is that the rat intestines secrete a phosphatase which is capable of hydrolysing sodium inositol hexaphosphate. Another interesting result of the investigation was the fact that vitamin D₂ failed to show any favourable effect upon the absorption of phosphorus from different sources, while Collip's Parathormone caused a considerable amount of this element to be taken up.

Similar results as to the hydrolysis of inositol phosphates were obtained by PATWARDHAN (1937), who extracted the responsible phosphatase from the intestinal mucosa of both adult and suckling rats. The pH optimum of the enzyme is in the region of 7.8. The reviewer must add that this value is probably never attained in the small intestines. A very important observation of PATWARDHAN must be emphasized, viz. that magnesium ions intensify the activity of the inositol phosphatase. Hence, results from experiments with »Phytin» cannot be generalized. PATWARDHAN concludes that a hydrolysis of inositol phosphates irrefutably takes place in the intestinal tract of albino rats, and that this hydrolysis is truly enzymatic in nature. It is brought about to a considerable extent by an enzyme secreted by the intestinal mucosa, and not exclusively by the vegetable phosphatases included in the cereal part of the diet. This conclusion, however, holds good for rats only. Intestines of guinea-pigs and rabbits yield inactive or only feebly active extracts.

Recently, MELLANBY and HARRISON (1939) have taken up the problem of the inositol phosphoric acid and the rhachitidogenic action of cereals to renewed experimentations, using puppies as experimental animals. They reject the hypothesis that the phosphorus of inositol phosphates should be especially unavailable to intestinal absorption, and, instead, focus the attention on *calcium* in this respect. They suggest the

possibility that inositol hexaphosphoric acid of cereal origin renders the absorption of this element more difficult than otherwise.

The present investigation did not have such a circumscript aim as most of those reviewed above. The object was to determine the general character of the metabolism of phosphorus from inositol hexaphosphate. The preparation used was the sodium salt, obtained from Messrs CIBA, Lmt'd. in Bâle. Its phosphorus content was determined by us and found to be 18.57 per cent, on a dry basis. Otherwise, as to general planning, the present investigation resembled the previous one of this series (WESTERLUND, 1939). As formerly, a deeply negative calcium balance was induced in the animals, brought about partly by a very restricted intake of this element, and partly by an abundant supply of tri-stearine. Furthermore, the diets were lacking in vitamins A and D. The purpose of these measures was to make the fecal calcium essentially endogenous in origin.

The Experimental Procedure.

The experimental animals consisted of 10 male albino rats taken from our own colony. They are referred to in the text by a letter and a figure. The letter denotes the food mixture, the figure the animal. The body weights at the end of every 24 hours period are given in Tab. 1. The bottom line shows the means.

Tab. 1.

The body weights of the animals in kilograms at the end of every 24 hours experimental period.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
.253	.223	.261	.221	.260	.263	.343	.349	.360	.406
.257	.225	.253	.221	.261	.263	.343	.346	.359	.407
.255	.225	.253	.225	.261	.263	.338	.342	.356	.402
.254	.229	.253	.223	.261	.264	.336	.343	.354	.400
.254	.229	.251	.223	.263	.268	.336	.343	.354	.400
.254	.230	.250	.221	.261	.268	.334	.340	.354	.400
.255	.227	.254	.222	.261	.265	.338	.344	.356	.403

The experiments ran for 10 days, the first four of which constituted the preparatory period. The actual test period, during which the excreta and the wasted food materials were collected and analysed, began with the 5th day and lasted 6 days. All figures in Tab. 1 and following relate to this test period. The pre-experimental treatment of the animals as well as their experimental cages were the same as in the previous investigation.

The experimental diets. The preparation of the food mixtures and the composition of the basal food, including the salt mixture used, were likewise the same as in the previous investigation. In the present case also, we tried to equalize the sodium content of the different diets by adding calculated quantities of sodium bicarbonate (NaHCO_3). Two rats received the same food mixture. As to the starch ingredient in the mixtures, it should be noted that it originated from a different batch in the food mixtures Nos. A, B and C than in the food mixtures Nos. D and E. As no attempts were made to remove calcium and phosphorus from the starch, differences in the calcium content of the two groups of food mixtures were to be expected. Every mixture contained 30 grams of tri-stearine.

The total food consumption of the individual rats is given in Tab. 2. A glance at the Table shows that the appetite

Tab. 2.

Total food consumed, dry substance in dekagrams.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
1.11	1.35	1.01	1.03	1.06	1.10	1.40	1.42	1.35	1.41
1.35	1.40	1.27	1.32	1.34	1.32	1.40	1.41	1.34	1.41
1.37	1.39	1.26	1.28	1.25	1.31	1.41	1.40	1.36	1.42
1.38	1.44	1.28	1.37	1.37	1.42	1.42	1.42	1.37	1.45
1.47	1.46	1.33	1.36	1.38	1.37	1.42	1.44	1.40	1.46
1.49	1.50	1.32	1.38	1.40	1.46	1.42	1.45	1.43	1.47
1.362	1.423	1.245	1.290	1.300	1.330	1.412	1.423	1.375	1.437

has been fairly regular from day to day during the experiment. An analysis of variance reveals that the variation between the animals has been significantly wider than within them.

The Accuracy of the Chemical Analyses.

The methods of chemical analyses were the same as described in the previous investigations of this series. Actual analyses were only made for Ca and P, the percentages of the other ingredients being obtained by calculation, on the basis of the weighed quantities of them and of the dry weights of the ready-made food mixtures. Consequently, the accuracy of the data for the consumption of the last-named ingredients is not so great.

The analyses obtained for *phosphorus*, reduced to a dry basis, have been tabulated in Tab. 3, which shows the inositol phosphate addition and the dry weights of the food mixtures as well.

Tab. 3.

The P percentages of the food mixtures.

	A	B	C	D	E
Phosphate added, g	2.53	1.25	1.90	4.96	3.76
Weight, g	326	320	315	336	336
P-percent.	.1508	.0938	.1154	.2972	.2369
	.1584	.0865	.1201	.2976	.2372
	.1530	.0872	.1252	.2968	.2364
Means	.15407	.08917	.12023	.29720	.23683

Concerning the starch, we divide the food mixtures in two groups, one including the diets A, B and C, the other the diets D and E. If the P percentages of the former are adjusted by regression to a similar addition of phosphate, and this new set of P percentages is put through an analysis of variance, we find that the variation within the food mixtures is wider than between them. The same results is obtained

also for the food mixtures Nos. D and E, regardless of whether we use the regression coefficient of the first group or use the value of it actually derived from them.

The accuracy of the original P determinations is illustrated in Fig. 1. It shows clearly the existence of two groups of P determinations. If now — by the aid of the regression coefficient for the first group — we calculate by extrapolation

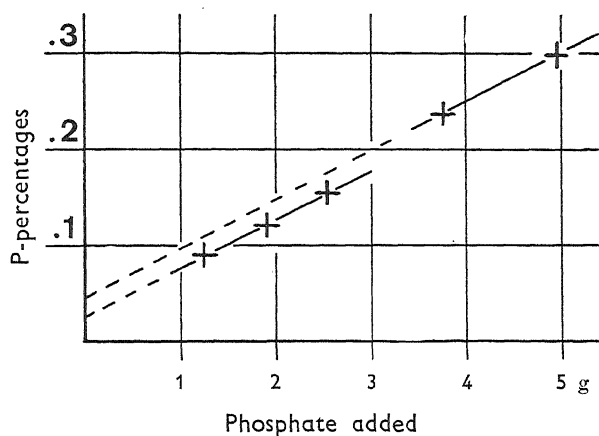


Fig. 1. The regression of the means of the P percentages in the individual food mixtures upon the phosphate addition. The same regression coefficient has been used for the two groups.

the values of P percentage in a food mixture without any addition of inositol P, we naturally obtain two values, one relating to the first group, the other to the second, or:

for the food mixtures D and E0457,
» » » » A, B and C0249,
ratio	1.84.

The analyses obtained for *calcium*, reduced to a dry basis, are given in Tab. 4. It shows that the Ca percentages in the food mixtures D and E are higher than in the remaining ones, and a numerical analysis shows that this difference is

highly significant. The means of the Ca percentages in the 5 food mixtures are as follows:

in D and E0254,
" A, B and C0137,
ratio	1.85.

The ratio attains the same value as for the extrapolated P percentages. This agreement confirms the accuracy of the determinations of P and Ca. Furthermore, it can be derived from Tab. 4, that the variation within the food mixtures is wider than between them, if both groups are taken separately.

Tab. 4.

The Ca percentages of the food mixtures.

A	B	C	D	E
.0117	.0120	.0146	.0263	.0251
.0178	.0142	.0133	.0256	.0249
.0110	.0146	.0140	.0254	.0252
.01350	.01360	.01397	.02577	.02507

But on the other hand, returning to the P percentages, if we *calculate* the P percentages in the different food mixtures on the basis of their dry weight and with due allowance to the extrapolated values in a food mixture without an addition of inositol P, we find higher values than those actually determined by chemical analysis. Therefore, in the preparation of the food mixtures, considerable losses of inositol P must have taken place. The mean of these losses amounts to 9 per cent, with a moderate variance.

The Problem.

The object of the present investigation, like that of the two previous ones in this series, was to present complete balance sheets for phosphorus and calcium at different levels of intake. Furthermore, it seemed desirable to seek for the factors which

determine the elimination of these elements with the excreta of the animals. The solution of this problem was brought about by the aid of numerical analysis, and in doing so extensive use was made of Student's *t* test (cf FISHER, 1930) as a criterion.

Phosphorus.

The renal elimination. The amounts of phosphorus, eliminated by the kidneys during periods of 24 hours, are given in Tab. 5. For the same reason as in the previous communication, only one value can be given for each rat. It denotes one sixth of the total urinary phosphorus from all six experi-

Tab. 5.

Mean values in centigrams for urinary phosphorus in the individual rats during 24 hours.

	a	b	c	d	e
1	1.697	2.083	1.939	4.097	2.689
2	2.554	1.747	1.693	3.979	3.059

mental days, and, consequently, represents a mean value. The method of obtaining these means excludes the knowledge of their numerical structure. Tab. 5 shows a considerable variation between the animals on different diets. This result was expected. More unexpected was the wide deviation between the two animals fed with the same food mixture. This is especially marked for diet No. A. A deviation small enough to be regarded as obtained at random is found for diet No. D only.

Because of the considerable number of observations, the numerical analysis was carried out in the usual way, i. e. by calculating net regression coefficients of the different concomitant variates after successive introductions of them into the system of the observation equations. The first variate introduced in this way was the daily consumption of phos-

phorus. These quantities are given in Tab. 6. A glance at it shows that the variation between the animals is much wider than within them.

Tab. 6.

The daily consumption of phosphorus, in centigrams.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
1.72	2.08	.90	.92	1.27	1.32	4.16	4.22	3.21	3.35
2.08	2.16	1.13	1.17	1.62	1.59	4.16	4.20	3.18	3.33
2.11	2.14	1.13	1.14	1.51	1.58	4.20	4.17	3.22	3.36
2.13	2.22	1.14	1.22	1.65	1.70	4.21	4.21	3.24	3.43
2.26	2.25	1.19	1.21	1.66	1.65	4.21	4.27	3.32	3.45
2.29	2.31	1.18	1.23	1.68	1.75	4.22	4.30	3.38	3.47
2.098	2.193	1.112	1.148	1.565	1.598	4.193	4.228	3.258	3.398

The calculation of the coefficient for the regression of the renal phosphorus elimination upon the phosphorus consumption results in a highly significant value. On the other hand, the regression line cannot be said to represent the observations in a fully satisfactory way. On the contrary, a systematic trend is observed in the difference between the observed values and those adjusted by regression. This trend is so marked that the question arises whether the observations would not be better represented by a curved line. This seems to be the case, for, if we use the formula

$$y = a_0 + a_2 x^2,$$

where y denotes urinary phosphorus and x food phosphorus, we obtain a value for a_2 which is 10 times its mean error. But an analysis of the significance of the two covariations proposed (one recti-linear, the other curvi-linear), using z as a criterion, shows but a small difference between the two values of z . Furthermore, there is a physiological reason against the possibility of the regression line being curved. Our line has the concavity upwards. A covariation of that kind does not seem to be in accord with known physiological facts, at least not within the fairly wide range of phosphorus consumption

which has been realized in the present investigation. A concavity downwards would be more resonable, as the intestinal absorption, and, consequently, the renal excretion must be assumed to approach a constant value with the character of an asymptote parallel to the abscissa. We, therefore, postulate that all the covariations discussed in the present communication can be represented by straight lines.

The continued introduction of concomitant variates gave the result that, aside from the phosphorus consumed, only two, viz. the fecal phosphorus and the fecal calcium, can be regarded as joint controllers of the renal phosphoric elimination. This result is shown in Tab. 7.

Tab. 7.

Net regresssion coefficients (P) of three variates, successively introduced into the system of observation equations, for covariation with the renal elimination of phosphorus.

	$P \pm \varepsilon(P)$	t	$P \pm \varepsilon(P)$	t	$P \pm \varepsilon(P)$	t
P consumed	$+ .70 \pm .10$	7.0	$+ .72 \pm .09$	8.0	$+ .87 \pm .11$	7.9
Fecal P			$- .65 \pm .48$	1.4	$- 2.57 \pm 1.02$	2.5
Fecal Ca					$+ 1.50 \pm .73$	2.1
Error	$\pm .36$		$\pm .33$		$\pm .29$	

Table 7 also shows, that the significance of the two additionally introduced variates is rather low. One detail, however, should be noted, viz. the considerably raised significance of fecal phosphorus caused by the introduction of fecal *calcium*. Such a result is sometimes found following successive introductions, when the introduced variates are correlated mutually. Such is the case here. The coefficient of the regression of fecal phosphorus upon fecal calcium is 3.4 times its mean error, and, therefore, must be considered clearly significant. The consequence of this is that the covariation between urinary phosphorus and fecal calcium may be regarded as a *covariatio*

spuria. The question may be settled by freeing the values of fecal phosphorus from the simultaneous variations of fecal calcium. If this is done, and the residuals thus obtained are introduced into the system of observation equations, together with the data for phosphorus consumption and for fecal calcium elimination as well, the net regression coefficients of Tab. 8 are obtained. This new calculation relieves the fecal calcium of all significance.

Tab. 8.

Net regression coefficients of three variates for covariation with the renal elimination of phosphorus.

	$P \pm s(P)$	t	$P \pm s(P)$	t	$P \pm s(P)$	t
P consumed	$+ .70 \pm .10$	7.0	$+ .86 \pm .09$	9.6		
Fecal P residuals			$-2.54 \pm .92$	2.8		
Fecal Ca					$- .03 \pm .31$.1
Error	$\pm .36$		$\pm .27$		$\pm .29$	

The conclusion to be drawn from this discussion is that only two concomitant variates show significant covariations with the renal elimination of phosphorus, viz. the phosphorus consumption and the phosphorus content in the fecal excreta, when due allowance is made for such parts of the last-named variate as covariate with the calcium content of the feces. The significance of the influence of fecal phosphorus is by no means high, as the corresponding probability is in the vicinity of .03. However, it may be considered of sufficient significance to show the tendency for a decrease in fecal phosphorus co-existent with an increase in the urinary phosphorus.

If now — by the aid of the last-mentioned regression coefficient — the values of urinary phosphorus are freed from the influence of fecal phosphorus, we get a correspondence between these residuals and the phosphorus consumption, which is illustrated in Fig. 2.

The regression line is practically a diagonal, as the regression coefficient for such a line (1.00) does not differ significantly from the regression coefficient actually obtained (.86). The diagonal cuts the y-axis at a value of .07 centigram, i. e. practically in origo. The conclusion to be drawn from this is, that above a basal value of .7 milligram, obtained by extra-

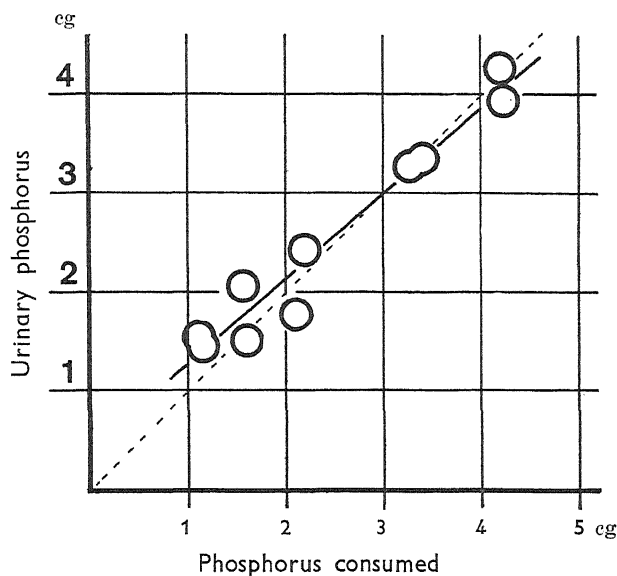


Fig. 2. The regression of urinary phosphorus upon phosphorus consumption, when other influencing factors are assumed to be constant.

pulation, the whole quantity of phosphorus consumed is recovered in the urine.

Fig. 2 shows that considerable differences between the observed values and those adjusted by regression still remain. If these differences are determined and algebraically added to the mean of the urinary phosphorus, the values in Tab. 9 are obtained. Consequently, they represent residuals of urinary phosphorus, i. e. freed from the influence of varying phosphorus consumption and varying fecal phosphorus elimination.

Tab. 9.

Mean values of urinary phosphorus in the individual rats during 24 hours in centigrams, when phosphorus consumption and fecal phosphorus elimination are assumed to be constant.

	a	b	c	d	e
1	2.10	2.73	2.85	2.80	2.60
2	2.67	2.59	2.27	2.41	2.52

Mean = 2.554

In order to strengthen the results in Tab. 8, one can test these residuals as to a covariation with other concomitant variates. Such a covariation with a significance to be spoken of cannot be demonstrated. Therefore, an account of the residuals in Tab. 9 cannot be given. They must depend on non-recorded factors.

The fecal elimination. The experimental data for the fecal elimination of phosphorus are given in Tab. 10.

Tab. 10.

The fecal phosphorus elimination, in centigrams, in the individual rats during 24 hours.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
1.14	.90	.27	.33	.94	.97	.61	.71	.60	.45
.79	.76	.30	.33	1.20	.70	.53	.68	.92	.49
.84	.85	.29	.24	1.28	.53	.93	.65	.68	.60
.75	.67	.27	.32	.99	.67	.63	.61	.59	.48
.69	.79	.32	.28	.93	.59	.54	.50	.54	.47
.67	.76	.32	.24	.90	.62	.57	.59	.97	.60
.813	.788	.295	.290	1.040	.680	.635	.623	.717	.515

The analysis of variance (Tab. 11) gives the difference σ a value of 1.5, whereas FISHER's Table VI for the 5 per cent point has a value in the region of .4, thus indicating that the variation between the animals is significantly wider than

Tab. 11.

Analysis of variance of the fecal phosphorus elimination.

Source of variance	Degrees of freedom	Sum of squares	Mean squares	S. D.	log. nat. 10 S. D.
within the animals	50	.7675	.01535	.1239	.214
between the animals	9	2.8621	.31801	.5639	1.730
total	59	3.6296	$z = 1.52$		

within them. A contributory cause to this result is the difference between two animals receiving the same food. These differences are given in Tab. 12. As shown there, the difference between animals Nos. c 1 and c 2 is especially conspicuous.

Tab. 12.

The differences in the fecal phosphorus elimination between the two animals on the same treatment, and the significance of these differences.

Difference between	centigram	t
a 1 and a 2	.025	.32
b 1 and b 2	.005	.25
c 1 and c 2	.360	3.97
d 1 and d 2	.012	.18
e 1 and e 2	.202	2.54
minimum		3.17

Among the variates which show a significant gross covariation with the fecal elimination of phosphorus, the co-existent elimination of calcium must be mentioned. The traditional numerical analysis reveals that one additional variate shows a certain tendency to such a covariation, viz. the corresponding consumption of phosphorus. The net regression coefficients of these two variates are given in Tab. 13.

Tab. 13.

Net regression coefficients (P) of two variates for covariation with the fecal elimination of phosphorus.

	$P \pm \varepsilon(P)$	t	$P \pm \varepsilon(P)$	t
Fecal Ca	$+ .59 \pm .13$	4.5	$+ .65 \pm .11$	5.9
P ingested			$+ .07 \pm .03$	2.3
Error	$\pm .13$		$\pm .11$	

The coefficient of the phosphorus consumption is of low significance; with 7 degrees of freedom the odds are only 95 to 5 that the coefficient is greater than zero. With the moderate demands on significance, which one is bound to put

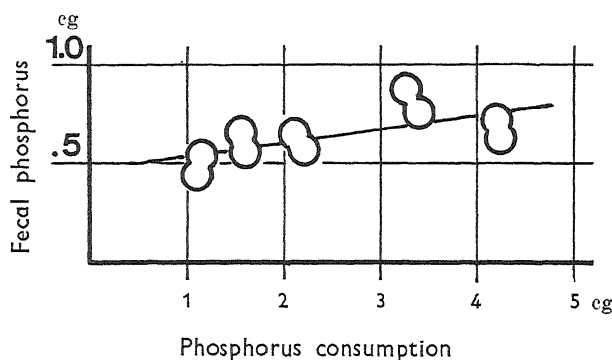


Fig. 3. The relation between phosphorus consumption and fecal phosphorus elimination, when the fecal calcium elimination is assumed to be constant.

on physiological experiments, these odds may be considered sufficient; the remaining variates are far from coming up to them.

As usual, we remove the influence of varying elimination of fecal calcium from the values of fecal phosphorus elimination, and get a correspondence between these residuals and the phosphorus consumption which is illustrated in Fig. 3.

Calcium.

The renal elimination. The experimental data are given in Tab. 14. They have been obtained in the same manner as the data for urinary phosphorus. They show a pronounced obliquity in the distribution. The most aberrant value belongs to animal No. c 2. The cause of its calciuria cannot be given. Annotations about the behaviour of the animal have not been made.

Tab. 14.

Mean values, in centigrams, for urinary calcium in the individual rats during 24 hours.

	a	b	c	d	e
1	.035	.026	.086	.064	.030
2	.101	.027	.255	.028	.030

The numerical analysis shows that none of the concomitant variates recorded exerts a significant influence upon the renal elimination of calcium, at least when all animals are included in the calculation. But if we exclude animal No. c 2, we find a feeble tendency to a covariation with the fecal calcium, as is shown in Fig. 4. The regression coefficient is only 2.1 times its mean error.

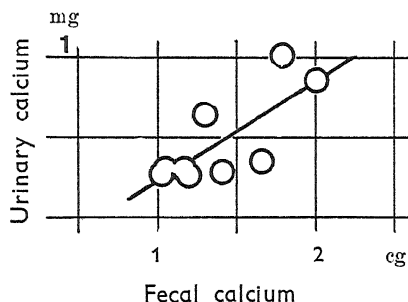


Fig. 4. The relation between the elimination of fecal and urinary calcium, when the values for animal c 2 are excluded.

Tab. 15.

Urinary calcium in per cent of the total calcium elimination.

	a	b	c	d	e
1	2.1	2.1	4.1	4.7	2.5
2	5.4	2.6	14	2.0	2.8

Finally, Tab. 15 is given, which shows the urinary calcium in per cent of the sum of urinary and fecal calcium. The singularity of animal No. c2 is obvious. Without its value the mean is 3.1 per cent.

The fecal elimination. The experimental data for this variate are given in Tab. 16. The analysis of variance gives the difference \approx the value of 1.6, while the 5 per cent probability requires .4 only. Consequently, the variation between the animals must be considered significantly wider than within them.

Tab. 16.

The fecal elimination of calcium, in centigrams, in individual rats during 24 hours.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
1.45	1.45	.88	.79	1.87	1.53	1.07	1.38	1.02	.84
1.48	1.48	1.31	1.05	1.43	— ¹	1.29	1.26	1.32	.97
1.81	2.04	1.09	.83	1.56	1.37	1.22	1.46	.99	1.24
1.58	1.43	1.19	1.20	1.95	1.77	1.46	1.39	1.05	1.04
2.15	1.93	1.36	1.12	2.31	1.39	1.43	1.32	1.10	1.07
1.50	2.21	1.38	1.18	2.96	1.94	1.34	1.62	1.56	1.12
1.662	1.757	1.202	1.028	2.013	1.600	1.302	1.405	1.173	1.047

The numerical analysis shows that two variates have significant covariation with the fecal output of calcium, viz. the fecal phosphorus and the phosphorus ingested. The significance of the lastnamed variate is rather low, as reflected in Fig. 5. The circles represent residual values of calcium, i. e. freed from the influence of fecal phosphorus. The power of the regression line to represent the observations satisfies moderate demands only.

¹ Lost.

Tab. 17.

The daily consumption of calcium in centigrams.

a 1	a 2	b 1	b 2	c 1	c 2	d 1	d 2	e 1	e 2
.15	.18	.14	.14	.15	.15	.36	.37	.34	.35
.18	.19	.17	.18	.19	—	.36	.36	.34	.35
.19	.19	.17	.17	.18	.18	.36	.36	.34	.36
.19	.19	.17	.19	.19	.20	.37	.37	.34	.36
.20	.20	.18	.18	.19	.19	.36	.37	.35	.37
.20	.20	.18	.19	.20	.20	.37	.37	.36	.37
.185	.192	.168	.175	.183	.184	.363	.367	.345	.360

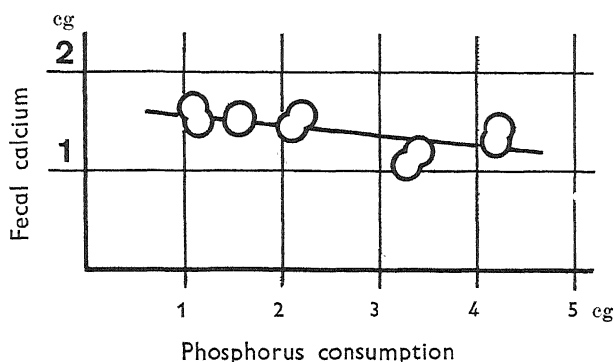


Fig. 5. The relation between fecal calcium and phosphorus consumption, when fecal phosphorus is assumed to be constant.

But the tendency of an increase in phosphorus consumption to cause a decrease in the fecal output of calcium must not be overlooked.

There is an interesting detail to call attention to in this connection, viz. the fact that a variation in the calcium consumption seems to be totally without any demonstrable influence upon the corresponding fecal output of this element. The experimental data for the consumption of calcium are given in Tab. 17.

The analysis of variance shows that the variation between the animals must be considered significantly wider than within them. The reason for this lies in the two kinds of starch

used. The animals which received food mixtures Nos. D and E consumed significantly more calcium than the animals receiving food mixtures A, B and C. The difference between the mean consumption of the two groups must be considered significant, it is true, but it is really very moderate, amounting only to 1.8 milligrams. Within this very narrow interval of calcium intake, any difference in the fecal output of this element is scarcely to be expected on such a low level of intake as has been realized in the present investigation (cf WESTERLUND, 1938).

The mean value for the calcium consumed by all the animals is 2.5 milligrams. If we compare this value with the corresponding value for the fecal elimination (14.2 mg), we find that the animals have excreted about 6 times as much calcium in their feces as has been consumed with the food.

Summary.

The present investigation is a continuation of the previous one with sodium phosphate, which has been replaced here by sodium inositol hexaphosphate. The experimental animals were 10 full-grown male albino rats. They were taken through an experimental period of 10 days, the last 6 of which constituted the test period proper. The investigation has given the following results.

(1) The *renal* elimination of *phosphorus* is determined by two factors, viz. (a) the oral consumption of the element, and (b), though to a considerably less extent, the fecal elimination of it. The last-named covariation is negative, i. e. an increase in renal excretion is followed by a decrease in fecal elimination and *vice versa*. If the fecal elimination of phosphorus is assumed to be constant at its mean value, the amount of phosphorus consumed is excreted practically quantitatively in the urine.

(2) The *fecal* elimination of phosphorus is likewise determined by two factors, viz. partly by the corresponding fecal elimination of calcium, and partly by the consumption of phosphorus. The latter covariation stands on the borderline to insignificance.

The former covariation is clearly significant and positive. It indicates that a heavy ingestion of calcium impairs the absorption of inositol phosphorus, and *vice versa*, as has been suggested by different authors.

(3) The *renal* elimination of calcium bears no relation to any one of the concomitant variates recorded. The distribution of the values is extremely oblique. The participation of the kidneys accounts for about 3 per cent of the total excretion, if the values for the most aberrant animal are excluded.

(4) The *fecal* elimination of calcium is determined to a moderate extent by the corresponding ingestion of phosphorus; it is also affected by the fecal elimination of phosphorus. An increase in phosphorus consumption is followed by a slight decrease in the fecal elimination of calcium. On the other hand, no relation between the calcium consumption and the corresponding fecal elimination of calcium can be demonstrated.

(5) The results of the present investigation provide no basis for the opinion that inositol phosphorus should be less available for intestinal absorption than the other phosphorus compounds previously studied. But a comparison in details of these compounds must be deferred to a special communication.

Sammanfattning.

Fosfor- och calcium-omsättningen hos fullväxta råttor med natrium-inositol-hexafosfat som enda fosforkälla och på calciumfattig näring.

Undersökningen utgör fortsättning på den föregående med natrium-fosfat, som här ersatts med natrium-inositol-hexafosfat. I denna form förekommer fosfor i avsevärd mängd uti spannmål. Det råder olika uppfattning om tarmkanalens förmåga att uppsuga denna fosfor-form. En uppfattning säger, att denna förmåga är ganska liten, och att inositol-fosfor alltså skulle ha låg smältbarhet. Om denna uppfattning är riktig, så skulle halten av total-fosfor, framför allt i kraftfoder, vara alldeles missvisande från näringsfysiologisk synpunkt.

Den här föreliggande undersökningen bestyrker icke den nämnda uppfattningen. Resultatet av densamma är att inositol-fosfor förhåller sig från omsättningssynpunkt på praktiskt taget samma sätt som natrium-fosfat-fosfor, d. v. s. att så gott som hela den förtärda mängden uppsuges av tarmkanalen och avsöndras med urinen. Det understrykes, att detta resultat vunnits med en näring, som varit ytterst calcium-fattig. Vissa literaturuppgifter tyda på att resultatet sannolikt blivit ett annat, om näringen varit mera calcium-haltig.

Eljes äro resultaten i stort sett desamma som i den föregående undersökningen. Bland dem bör särskilt framhållas, att en ökning av *fosforkonsumptionen* i någon mån medför en minskning av den mängd *calcium*, som förloras med tarmutömningen.

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Investigations as to the absorption and accumulation of inorganic ions.

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From the Institute of Plant Physiology.

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Introduction.

This paper deals with experiments performed with the object of elucidating the problem of the active uptake of salts in the living organisms. The results support a new conception of the properties of the protoplasmic surface, and the elements of an electro-chemical theory of the salt absorption are set forth.

In order to facilitate the presentation of certain new ideas and to avoid tiring recapitulations, the following nomenclature is used.

Bulk phase = the environment of the cell (for roots = the nutrient solution).

Protoplasmic membrane = the aggregate of the oriented and non-oriented molecules which constitute the outer layer of the protoplasm.

Surface layer = the layer of probably oriented molecules (perhaps a »monolayer«), which is in contact with the bulk phase.

Sublayer = the inner side of the membrane, which is probably the site of chemical processes coupled to the absorption mechanism.

Aqueous film = the layer of the bulk phase which is directly influenced by the ion exchange and CO₂ deliberation. Frequently = the moist cellulose membrane and adjacent layers of the substrate.

O-boundary = the boundary between the protoplasm and the bulk phase.

O-level = the non-accumulated stage of salts (for roots = the solution).

I-level = the accumulated stage of salts. In single cells = the cell sap, in multicellular organs more undecidedly = the interior of the organ, or the part of the organ in which salts are accumulated.

Z = a prefix which indicates the surface of the protoplasm. E. g. ZcH = the hydrogen ion concentration in the surface layer, ZcM = the concentration of metallic cations in this layer. Corresponding symbols are ZpH etc.

L = a prefix which indicates the bulk phase, or the outer side of the O-boundary. E. g. LcH = the hydrogen ion concentration of the bulk phase, LcM and LcA = the concentration of metallic cations or anions of acids in the solution etc. Corresponding symbols are LpH etc.

In this paper the conception »concentration» always refers to ideal conditions, and due reservation ought to be made for the transference into activities. In extending the conception concentration to surfaces reservation must also be made in respect of special surface forces, which affect the activities of the ions. In the following discussions the interphases in a boundary are conceived as ideal sections through a uniform solution. This is of course only a rough approximation, but a theory of activities of layers, which are built up by oriented molecules, is not yet developed.

1. General characteristics of the protoplasmic membrane.

The majority of research workers in permeability have restricted themselves to the study of the uptake of uncharged molecules in the living cell. The discussion as to the chemical and physical nature of the protoplasmic membrane has yet not settled. Lipoids, perhaps phosphatides, seem to be constituents of the membrane, but the entrance of molecules is not exclusively controlled by the solubility in lipoids. Also the »porosity» of the membrane seems to be a factor of importance (see discussions in WILBRANDT 1938). According

to the general opinion of investigators of permeability water, soluble molecules and ions move more or less retardedly through the »pores», and their diameters determine the velocity of entrance. Molecules which are soluble in fats, are supposed to be dissolved in the interspaces between the »pores».

OSTERHOUT (1935, 1936 and several papers in J. gen. Physiol.) has presented a somewhat divergent opinion as to the properties of the protoplasmic membrane. He claims for it a »non-aqueous» character. This hypothesis leads to the conclusion that molecules and ions do not pass through the membrane together with water molecules. OSTERHOUT develops the conception that molecules of a substance which is dissolved in the bulk phase, are transferred into the »non-aqueous» membrane in a new dissolved state. The velocity of entrance is supposed to be controlled by the partition coefficients water: protoplasm. OSTERHOUT denies the existence of free ions in the membrane. According to his ideas, ions of neutral salts are absorbed as neutral molecules or ion pairs. Of the salt M.A the ions will combine with the ions of the water into M.OH or H.A respectively, and these neutral ion pairs (or molecules) will then be dissolved in the membrane just as are molecules of non-dissociated substances. In other papers OSTERHOUT develops a modified theory, according to which the cations combine with some compound in the membrane substance into a product M.R (if R is the hypothetical substance), which is split up again on the inside of the membrane.

OSTERHOUT's ideas as to the partition coefficient water: plasma as a controlling factor for the entrance of non-dissociated substances is no doubt related to OVERTON's lipid hypothesis. Many earlier and more recent investigations prove the importance of such apprehensions. The difficulty begins with the dissociated substances. OSTERHOUT starts from a very important observation. He has measured the electrical resistance of the thin protoplasmic layer in cells of *Valonia*. It amounts to c. 10000 ohms per cm^2 . In *Nitella* and *Chara*

the resistance is even higher, c. 250000 ohms per cm^2 (OSTERHOUT 1936).

If these observations have a general bearing, the cause is no doubt a low dissociation in the membrane. On the other hand, experience shows that the membrane is permeable to water. If the water passes in the usual way through »pores» in the membrane, ions too ought to be able to accompany the water molecules. It seems to me that the only possibility to escape from this contradiction is to assume a special structure of the membrane, similar to the organization of monomolecular layers (LANGMUIR 1939 and earlier papers). In a monolayer the molecules lie close together. If these molecules are partly dissociated, the hydrophilic ends invade the boundary between the layer and the water and develop an electrical charge which greatly restricts the passage of ions. A monolayer of fatty acids will, according to RIDEAL (1925), let molecules of water pass through it. The electrical resistance of such a layer must nevertheless be large.

The assumption of electrical charges, carried by dissociated, oriented molecules of the protoplasmic membrane is supported by a number of experiments, which we shall describe in detail later. The magnitude and distribution of the charges will largely condition the properties of the membrane, e.g. its permeability to water and dissolved molecules. A large number of investigators (e.g. DELLINGHAUSEN 1933, AMLONG and BÜNNING 1934, DE HAAN 1935, SCHMIDT 1936) have found that the permeability to water and dissolved molecules is influenced by neutral salts. If the membrane carries electrical charges, salt ions will of course alter these charges (see below) and hence alter the hydration of the membrane molecules, their mutual distance etc. An electrically charged membrane involves exchange of ions (e.g. H-ions against M-ions). As a consequence, the physico-chemical properties of the salt ions will be partly carried over to the membrane. It is a well known fact that K-ions have an effect upon the properties of the protoplasm quite different from that of Ca-ions. The »condensing» effect

of calcium and the «swelling» effect of potassium have been studied in the protoplasm (HANSTEEN-CRANNER 1922, SAKAMURA & KANAMORI 1935 and many others) as well as in soil colloids (LUNDEGÅRDH 1932), in the «auto-complex systems» of phosphatides (BUNGENBERG DE JONG 1935, 1938), etc. In all these cases changes in the charge of the colloids — or gelatinous membranes — will play a dominant role. This is not the place to give a detailed survey of permeability problems, so I restrict myself to touch upon the fact that the assumption of the existence of oriented, partly dissociated molecules in the protoplasmic surface will throw light upon a number of observations.

Previous work in permeability deals more or less exclusively with the function of the protoplasmic membrane as a filter or a solvent, respectively for molecules. Most investigators in this field do not occupy themselves with the forces which regulate the one-sided movement of dissolved substances from a solution into the cell or from one cell to another. Permeability experiments are usually performed with high concentrations of substances, and for this reason diffusion is usually claimed as the motive power. But even if a diffusion gradient exists, other forces cannot be excluded *a priori*. The diffusion movement is extremely slow. Research work in the migration of organic substances within a plant shows that the movement is frequently very much faster than it would be if diffusion were the motive power (MASON & PHILLIS 1937, CRAFTS 1939). It is a fact that roots absorb glucose from very diluted solutions, though they contain glucose derived from the leaves (see many experiments in the following pages). We speak here of non-dissociated substances, and we find that there are cases where these are moved against a diffusion gradient. The researches of ARISZ (1938) show that the uptake and transport of asparagine is coupled to respiration processes. Thus, other motive forces than diffusion seem to be available in the cell for the transport of certain non-dissociated substances.

If, now, the metabolism controls the uptake of a substance, the permeability problem will change. The metabolic activity can be asserted to be the first step in the uptake, i.e. the contact between the substance and the protoplasmic boundary, or it can enter later, after the substance has been «drawn» by diffusion through the membrane. It will be difficult to decide between these theoretical possibilities, and here I only wish to call attention to the fact that the velocity of the entrance of a non-dissociated substance in the cell cannot simply be used as a measure of the »permeability coefficient». And this coefficient is not a quite safe basis for conclusions as to the physico-chemical properties of the protoplasmic membrane.

If diffusion plays a somewhat doubtful role in the absorption and transport of molecules, it seems to have even less influence on the absorption of ions. It is a well-known fact that single cells, e.g. *Chara*, *Nitella*, *Valonia* (COLLANDER 1936, OSTERHOUT 1936), or roots accumulate inorganic ions from very dilute solutions against the diffusion gradient. The fact that neutral salts in diluted solutions are completely dissociated brings to the fore the question of how they traverse the membrane. OSTERHOUT claims that the cations and anions in contact with the membrane combine into neutral pairs and are dissolved as molecules in the »non-aqueous layer». But this hypothesis is open to criticism (cf. WILBRANDT 1938).

If the cell or organ is placed in contact with a solution of a single salt, full equivalency in the absorption of cations and anions is an exception. The cations and anions of the salt are usually absorbed with remarkable independance. Thus NO_3 -ions might be absorbed approximately at the same speed from $\text{Ca}(\text{NO}_3)_2$ as from KNO_3 (LUNDEGÅRDH 1937, p. 116, table X) whereas K^+ is much more quickly absorbed than Ca^{++} . These experiments show that the salt is not taken up as molecules by the combination of the metallic cation with the anion of the acid. Experiments with varying cH in the solution show, furthermore, that NO_3 is not absorbed as an ion pair with H^+ because the NO_3 -absorption is to a certain

extent independent of the cH (the probability of the ion combination $H^+ - NO_3^-$ varies of course directly with the cH and would, according to OSTERHOUT severely affect the anion absorption).

Experiments on salt absorption at constant LpH are difficult to perform, because of the well-known regulating power of the roots on the acidity of the solution. In combining the same anion with different cations, the LpH-value can be gradually changed, so that after 20 hours rather different end-values are attained. If the anion absorption is plotted against these pH-values, one finds an approximately constant absorption between pH 6 and 4.5 (LUNDEGÅRDH & BURSTRÖM 1933 b, p. 247, fig. 4). With higher pH-values a certain decrease ensued. New experiments by BURSTRÖM (1940) confirm these results as to the insignificant effect of LcH on the anion absorption.

I made two comprehensive series of experiments with phosphate solutions and 4-weeks-old wheat plants, growing in »photo-thermostats» (cf. description in LUNDEGÅRDH 1932). Absorbed quantities of anions and cations were determined every day for three days. In table 1 the total amount of absorbed ions is shown.

Table 1.

Absorption from phosphate solutions.

A. Potassium phosphate (mixtures of primary and secondary).

pH of solution		absorption in millimoles	
start	end	potassium	phosphate
4.8	5.1	0.148	0.227
5.6	5.5	0.346	0.187
6.0	5.6	0.229	0.164
6.5	6.0	0.138	0.188
7.0	6.5	0.162	0.193

Because of the buffer effect of phosphate, the LpH -values are comparatively constant here, whereas a certain levelling is noticeable. The phosphate absorption is practically constant in the region $\text{pH} = 5.5$ to 7.0 . The increase on the acid side is very gentle. Hence the full experience of this laboratory points in the direction that the effect of the hydrogen ion concentration on the anion absorption is less prominent. Also in respect of the cations the results by no means reflect simple physico-chemical principles. Table 1 shows a maximum at $\text{pH} = 5.5$, and a decrease at higher pH -values. Earlier results, too, are similar (LUNDEGÅRDH 1932, p. 172, table 33, many series). Generalization is nevertheless not advisable. Thus BURSTRÖM and BORATYŃSKI (1936) found that the absorption of Cu increased with the pH of the solution. LUNDEGÅRDH and BURSTRÖM (1933 b, p. 244, table IV and V) found a displacement in the balance anions: cations at the absorption from solutions which were made more or less acid by regulating the CO_2 -pressure. These results were explained by the Donnan equilibrium, according to the formula

$$\frac{[\text{H}^+]_o}{[\text{H}^+]_i} = \frac{[\text{M}^+]_o}{[\text{M}^+]_i} = \frac{[\text{A}^-]_i}{[\text{A}^-]_o} \quad (1)$$

where the appendixes o and i refer to the solution and the roots respectively. It ought to be mentioned that in some cases OSTERHOUT found a direct relation between cH in the solution and the absorption of an acid in *Valonia*-cells, but in other cases did not succeed in finding such a relation (JACQUES 1934).

Summarizing our experiences, I must conclude that it seems little probable that the anions enter the cell in combination with H -ions. If any simple relation between the cH of the solution and the ion absorption exists, this is most probably due to the membrane equilibrium effect, according to formula (1). This effect has a thermo-dynamical basis and is, therefore, found under all circumstances, whatever the mechanism of absorption may be. Experience shows, however, that the

Donnan-effect is realized only to a limited degree in the actual absorption. It is apparently faded by other factors.

There are a number of facts speaking against the opinion of OSTERHOUT and in favour of the theory *that inorganic ions are absorbed by means of exchange reactions at the membrane surface*, in addition to those mentioned above.

The ion antagonism involves interactions of cations (and perhaps also anions) already in the first stage of absorption. Investigations by BURSTRÖM (1934) show that the magnitude of the relation $\frac{M_1}{M_2}$ (M = cation) between two cations is of fundamental importance for the development of antagonism and that similar rules hold for cation adsorption in inorganic gels.

Another side of the ion competition at the cell surface is the exchange of cations observed in roots growing in solutions rich in Na or Ca. If these roots are put into single salt solutions of a potassium salt, K ions go in and a considerable amount of Na or Ca ions go out. This observation is a direct proof of the fact that a stage of absorption of cations is exchange with other cations, »adsorbed» in the organ surface. If the roots do not contain a surplus of metallic cations H-ions will function as exchange subjects: the cell is in the position to produce H-ions as well as OH- and HCO_3^- ions as long as metabolism continues. The exchange with H-ions is probably the normal primary absorption of cations from nutrient solutions. The existence of such exchangeable H-ions in the surface of the protoplasm is shown by observations of the surface potential in different solutions (LUNDEGÅRDH 1938, 1939). A brief summary of the results will be given here.

The method used for the potential measurements was quite simple: A 5—6 cm long piece of the growing root end of a wheat plant was placed between two solution-electrodes (see fig. 1). As upper electrode was used a capillary tube, into which the cut end of the root was introduced. One can, for

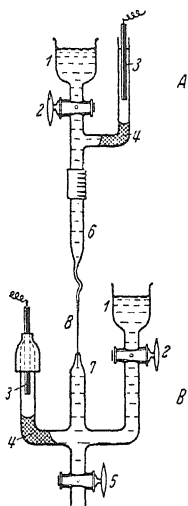


Fig. 1. Apparatus for the measuring of surface potentials of single roots. The upper vessel (6) contains 0.01 n KCl. The root (8) is held by the capillary end of 6 and its apical part is introduced into the lower vessel (7). Both vessels are provided with funnels (1) and cocks (2) for the filling, and with Zn-Hg-ZnSO₄-electrodes, which are connected with the galvanometer device. In new experiments the vessels (6, 7) are separated from the metallic electrodes by tubes with KCl-agar (replacing the KCl-gelatine plugs 4) and the potential difference between 6 and 7 is measured by the aid of a cathode-ray oscillograph.

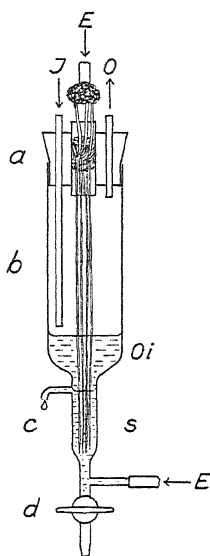


Fig. 2. Apparatus for the measuring of potentials of root systems. The seedlings are held by a glass tube in the rubber stopper *a*. On the cut stalks is placed a tuft of cotton wool soaked in 0.01 n KCl. For the electrical contact serve KCl-agar electrodes at *E*. The ends of the roots dip into the solution *s*. *c* is an outlet for constant level. If anaerobic conditions are wished, a layer of liquid paraffin (*oi*) is poured on *s*. Gases can be let in and out *b* by means of the tubes *J* and *O*.

the same reason, also use a whole seedling, the leaves of which are cut off 1—2 cm above the seed. As electrode can be used in this case a tuft of cotton wool which is soaked in salt solution. As the lower electrode can be used an open vessel containing the solution (see fig. 2). Both electrodes are connected by means of tubes with KCl-agar to non-polarized zinc

electrodes, which close a galvanometer circuit. By means of a compensation circuit and a bridge arrangement, the potential difference between the upper and the lower part of the root was determined.

If, now, the upper electrode was held constant (a 0.01 n KCl solution was generally used), and the solution surrounding the lower end of the root is changed, the voltage also changes almost instantaneously and then remains comparatively constant for several minutes. This fact proves that *the site of the potential difference is primarily the boundary between root and solution*, which is called the O-boundary in the following pages. In very diluted strong acids — independently of the nature of the anion — of a concentration of 0.001 to 0.00001 n, the root behaves approximately as an H^+ -electrode: a change in the concentration of one tenth power changes the potential by c. 58 mv, according to the formula

$$-E = \frac{RT}{nF} \ln \frac{ZcH}{LcH}, \quad (2)$$

where ZcH = the H -ion concentration at the surface of the root, LcH = the H -ion concentration in the bulk phase, T = the absolute temperature, R and F constants and n = valency (cf. fig. 3). Hence the electro-chemical behaviour of the root indicates the existence of a layer of H -ions in the surface. Several observations, e. g. the ceasing of the potential if the root is killed, show that *the cellulose membrane does not participate in these electro-chemical reactions*. The active surface is probably identical with the outer surface of the protoplasm of the epidermis cells. Similar results were obtained as well with the root tip as with the zone of young root hairs. The charge of the protoplasmic surface seems, then, to be a general characteristic of the root cells.

Measurements of the boundary potentials of an organ are always complicated by the fact that two boundaries are involved: one at each electrode. Furthermore, there are in the interior of an organ, as well as in single cells, a number of

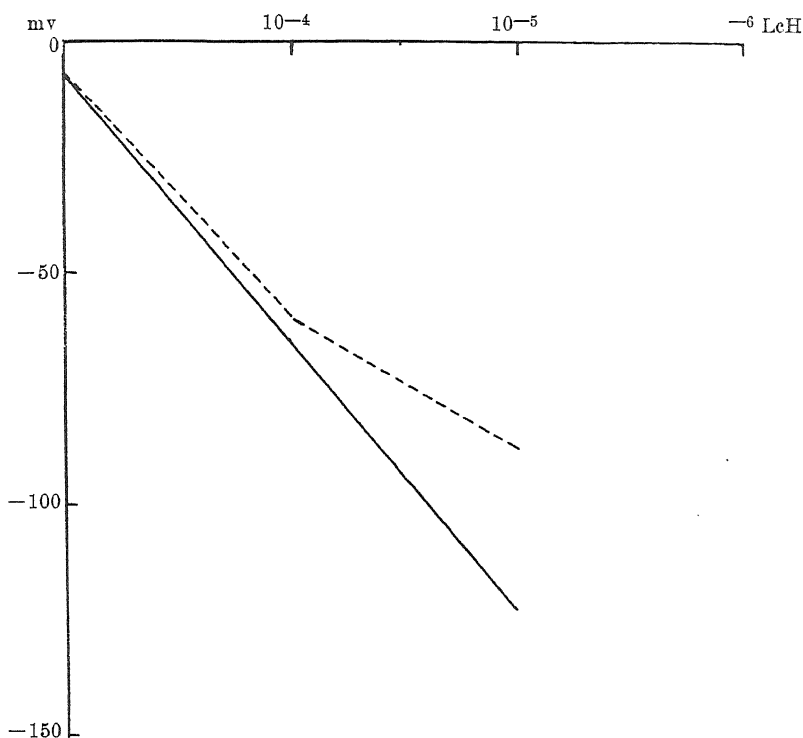


Fig. 3. The straight line represents the surface potential of wheat roots in pure solutions of mineral acids. The line corresponds to formula (2). The dotted line represents some deviations observed in cultures with low calcium concentration.

electro-chemical boundaries not only between protoplasm and outer solution, but also between protoplasm and cell sap, between protoplasm and nucleus, etc. It is to be expected that the »micro-boundaries» in single cells to a large extent eliminate one another in an organ, because the cells are placed wall by wall, coupled as electrolytic condensers. On the other hand, if cells of different anatomical specialization are joint, electromotive forces might appear. These internal forces are of course not influenced by sudden changes in the surface boundaries: As mentioned above, the measurement of the

immediate effect of a change in the solution will therefore reveal the *reaction of the surface boundary*. With the aid of special precautions it is possible to eliminate approximately the influence of the potential differences at the upper end of the root (LUNDEGÅRDH 1938 a), so that the measured electromotive force in the circuit can be used for the calculation of the approximative hydrogen ion concentration at the protoplasmic boundary of the root tip or any other part of the lower end of the root. For this calculation the formula (2) is used, with due reservation for using concentrations instead of activities and applying general kinetic principles to surface reactions. The surface potential of wheat roots corresponds to a $Z_{pH} = 3$ to 3.5. Oats give values similar to those for wheat. For barley roots somewhat higher values, $Z_{pH} = c. 4$ are obtained, and it is to be expected that the Z_{pH} value of the absorption zone of roots is a species character.

The behaviour of wheat roots in diluted acids shows now, as mentioned above, that the stability of the Z_{cH} -value ceases at L_{cH} values above $10^{-3} n$. This L_{cH} -value corresponds to the zero value of the root potential $\left(\frac{Z_{cH}}{L_{cH}} = 1\right)$. In acids stronger than the normal Z_{cH} value $\left(\frac{Z_{cH}}{L_{cH}} < 1\right)$ the root potential shifts over from negative to positive. This entails a disorganisation of the protoplasmic membrane and causes rapid death.

Here I again call attention to the important fact that the root potential in a diluted strong acid is independent of the nature of the anion. Curves identical with the straight line in fig. 3 were obtained with the following acids: HCl , HNO_3 , $H_2SO_4/2$, $H_3PO_4/3$. If the potential were a diffusion potential, it would be expressed by the formula

$$-E = \frac{U - V}{U + V} \cdot \frac{RT}{nF} \ln \frac{c_1}{c_0} \quad (3)$$

where U = the migration velocity of cations, and V = velocity

of anions, c_1 and c_0 the concentrations of the acid on both sides of the membrane. If U represents the H-ions of the acid and V its anions, $-E$ would be different in different acids, which is not the case. From the experiments with diluted acids the important conclusion must for this reason be drawn that only the H-ion concentration in the phase boundary causes the potential and that the surface of the living root holds negative valencies which bind H-ions.

As regards the absolute ZcH -value, calculated from formula (2), it must be considered to be only approximative, for the presumption that in our experiments the upper phase boundary of the root is uncharged holds only with an approximation of some 10 mv. A confirmation of our results that the surface of the protoplasm shows a markedly acid reaction is given by YAMAHA's (1938) determinations of the »iso-electrical point» of the protoplasm. He finds values between $pH = 3$ and 4.5.

A direct confirmation of the results gave measurements of the electro-osmotic behaviour of the root hairs.¹ Owing to the charge of the surface of the root hair this bends towards one of the electrodes, if the hair is exposed to a gradient of some 10—100 volts per cm. The deviation is directly proportional to the charge and attains zero at $ZcH = c. 10^{-3}$, which gives a direct confirmation of our calculations of ZcH_{max} .

To sum up: The previous conception of the protoplasmic membrane as an amorphous colloid does not tally with the new facts concerning the electrical charge and the individual ion absorption. New researches support the idea that *the membrane is built up of oriented molecules (cf. monolayer), some of which are dissociated on the acid side so that loosely-bound H-ions are scattered over the surface. These surface-bound H-ions give the membrane a negative electrical charge in solutions which are less acid than the membrane. If the solution is more acid than the membrane, the stability of the latter ceases,*

¹ Unpublished results.

and the cells are damaged. The first stage of cation absorption is to be assumed as *an exchange against the »adsorbed» H-ions.*

Determinations of the potential of the root in diluted acids rendered in 1938 and the first part of 1939 always values which indicated that the ZcH -value remained practically constant between $LcH=10^{-3}$ and 10^{-5} . The curve (see fig. 3) was a straight line with a slope of approximately 58 mv per pH-unit. I concluded from these results (LUNDEGÅRDH 1938, 1939) that the dissociation of the acid part of the protoplasmic membrane, from which presumably the ZcH -value arises, was not influenced by changes in the LcH -value within the named limit. If we presume that the acid reaction of the membrane is due to an acid, this acid will then be completely dissociated at least down to $pH=3$. From the general type of dissociation curves (see fig. 4, left curve) we may conclude that the presumed acid has a pK -value not higher than c. 1, which corresponds to a comparatively strong acid.

In accordance with general properties of the organical compounds which are thought to participate in the formation of the protoplasmic membrane, and owing to special observations (cf. p. 268), we are entitled to presume also a base dissociation in the membrane. This dissociation will be diminished if the LpH -value rises above a certain limit (fig. 4, right curve).

The balance between cH and cOH makes that a regression of the base dissociation will tend to rise the surface potential so that in this case an increase of the LcH -value by one tenth power would rise the potential more than 58 mv. As such an increase of the potential was not observed at $LcH=10^{-5}$, we can conclude that the pK -value of the presumed base component of the protoplasmic membrane is not lower than c. 7 (see fig. 4, right curve, cf. the dotted curve), but probably much higher, corresponding to the pK of a strong base.

Even if the base component of the protoplasmic membrane

is present only in a small amount in comparison to the acid component, its dissociation would nevertheless to a measurable degree influence the potentials. Several facts, e. g. the pronounced acid reaction of the membrane at $LcH=10^{-7}$ speak in favour of the assumption that the base component has a much lower concentration than the acid one.

The different concentration (activity) of the acid and base components of the protoplasmic surface makes the position of

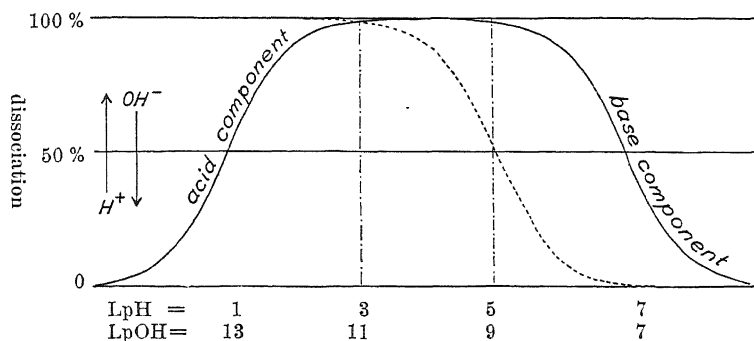


Fig. 4. Supposed dissociation curves of the acid and base component of the protoplasmic membrane. The position of the curve for the acid corresponds to $pK_a = c. 1$ (see p. 248). The approximately constant ZcH -value between $ZpH = 3$ and 5 gives the conclusion that pK_b is not lower than 7 , as the figure shows. The dotted curve shows that a lower pK_b -value would result in a varying ZcH -value in the named region.

the isoelectric point (i. e. p.) uncertain. In presence of a strong base and a strong acid no real i. e. p. will occur, but a rather broad isoelectric state, covering some two or more pH-degrees (cf. fig. 4). If the strong base is in minority this state will be moved against very low pH-values (probably below 2). This fact makes the importance of an isoelectric state of the protoplasm rather imaginary and explains why different investigators as a rule obtain so varying values.

The isoelectric »point» is of course quite different from the *isopotential point* (i. p. p.). The latter is attained when $ZcH = LcH$. Owing to the fact that ZcH depends on the concentration of cations in the bulk phase (LcM) all trials to de-

termine the »pH-value» of the protoplasmic surface are futile, as long as due attention is not paid to the LcM-value in each case. See below.

A series of determinations of ZcH_{\max} in the autumn 1939 rendered values which showed a slight decrease at $LcH=10^{-4}$ and a larger decrease at $LcH=10^{-5}$ in comparison to the earlier values. The decrease of ZcH was also in this case independent of the anions of the acid. The dotted curve in fig. 3 shows one of the cases, in which the deviation was comparatively large. On an average the decrease of ZcH at a jump of LcH 10^{-3} to 10^{-5} amounted to c. 0.5 pH-units.

The cause of this diverging behaviour of the roots is not quite clear. As ZcH in this case decreases with decreasing LcH the interference of dissociation is improbable, because the presence of weak acids or weak bases would both tend to increase the ZcH -value at decreasing LcH -values. The most probable explanation seems to be a reduced resistance of the membrane to high potential differences. Observations (p. 265) show that the calculated highest surface potentials are never realized. If the membrane is built up partly of oriented molecules these will, if the boundary potentials grow too high, partly turn over and eliminate the potential. Morphologically this means a partial »disorganization» of the membrane. In our earlier experiments this self-regulation of the potential occurred at 120—150 mv. The new sortiment of seeds, which was used since the summer 1939, seems to give more sensitive protoplasts, so that the partial elimination of the potential differences occurs already at lower potentials. A confirmation of this explanation quoted experiments with higher Ca-concentration in the nutrient solution. Calcium hardens the membrane (cf. p. 238), and the ZcH -values attained also nearly the theoretical values in these roots. In all other respects the new sortiment of roots behaved identically with the earlier ones.

2. The scope of absorption methods.

In studying one special function of the living organism, it seems appropriate to choose as the research object an organ which is exclusively built for that function. The roots of grass plants have a strong absorption power and it involves no difficulties to cultivate them from genetically pure lines under constant light and temperature conditions. Less suitable for absorption studies are slices of storage tissue, because their ion absorption comprises only a minor part of the total metabolism. In using roots it seems to be important that these are not minced and submerged in the solution (as is done by HOAGLAND & BROYER): The root is a pumping machine which pumps in salts into the lower parts (= region of the root hairs), transports them upwards and pumps them out again at the top of the root. If a detached root is surrounded by solution, a certain part of the absorbed salts will therefore escape again from the cut end. Some authors (cf. OSTERHOUT) claim that isolated large cells of algae are the best objects for the study of cell functions. I do not quite agree with this opinion: as a rule algae have a rather slow absorption power. The cells of *Valonia*, *Chara* and *Nitella*, which are generally used in such experiments (by OSTERHOUT and his school), are by no means physiologically simple, because they are not specialized, but combine absorption, carbon dioxide assimilation etc. in the same cell. This complication of the metabolism in association with the slow absorption of salts makes such cells less suitable for the study of the absorption mechanism in its relation to metabolism. In respect of ion absorption the root functions as a »symplast» (MÜNCH 1930). No chlorophyll function complicates the respiration, and roots which are isolated from vigorously assimilating plants contain enough sugar for at least 6 and frequently up to 20 hours of normal life (LUNDEGÅRDH 1937).

The absorption power of the root surface is, of course, not

uniform. The tip absorbs far less than the region of the root hairs, and this region more than the older parts.

The distribution of some cations in the whole root system is shown in table 2.

Table 2.

Distribution of some cations in the root system of wheat. The plants were 30 days old, with a length of roots of c. 20 cm. The total root system of 15 plants (containing c. 150 roots) was cut into pieces of 5 cm. and analyzed spectrographically after drying and extraction in 2 M HCl.

The figures represent millimoles per g dry weight. Two parallel series.

Zone	K		Ca		Mg		Mn	
0—5 cm (tips)	0.95	0.84	0.142	0.095	— —	— —	0.028	0.033
5—10 cm	0.75	0.62	0.066	0.067	— —	— —	0.020	0.022
10—15 cm	1.04	0.69	0.067	0.044	— —	— —	0.024	0.016
15—20 cm	0.91	0.84	0.041	0.037	0.06	0.06	0.022	0.015

The table shows that the alkali ions are approximately uniformly distributed in the roots, except for a weak minimum at a height of 5—10 cm. The calcium ions move more slowly and are for this reason retained in the tips. Mn-ions too are to some extent more accumulated in the tips. The distribution of the ions is of course a resultant of absorption and translocation. A comparison between ash analysis and analyses of the bleeding sap shows that the cells generally hold higher concentrations back than are transported (see below). This is more pronounced for ions of higher charge and lower mobility.

Probably differences also exist in the balance between absorbed cations and anions for different regions. We will return to these questions later. The differences in the velocities of ion absorption in different regions of the root

are probably due to correspondent differences in the metabolic activity and not primarily to the nature of the protoplasmic membrane. Potential measurements show that even in the root tip the membrane (0.5—1 mm from the summit) shows a H-ion activity of similar magnitude to that in the region of root hairs.

A certain degree of differentiation thus exists in the root, and it is scarcely possible to isolate the function of the cells, chiefly engaged in absorption work, from those which are more indifferent in this respect. It is also to be noticed that absorption in the sense of accumulation is one side of the transport process too, because transport of salts in a tissue means absorption power from one cell to another. It is very difficult to judge of a possible specialization of root cells in the absorption-accumulation work, performed from the outside, and transport work, effected from one cell to another cell. The epidermis cells may of course be able to perform the chief work, if they accumulate the salts at the outside and give them off at the inside: this would postulate a »polarity» within the cell itself. But it seems more feasible to adopt the »symplast» hypothesis and assume that the whole tissue in the region of the root hairs takes active part in the accumulation process, so that salts are pumped in through the epidermis and pumped out again in the central system of large cells which, upwards, are connected to the vascular system. In either case the accumulation means osmotic work from one O-level (= the solution) to one I-level (= the ascending aqueous sap) and the mechanism ought to be the same whether this work is performed in one single cell or in a »symplast».

Because of the difficulty in isolating the region of most intensive absorption-accumulation work in the root¹, the study

¹ GREGORY and collab. (1939) have adopted a similar method to that of SIERP & BREWIG (1935) in studying the absorption power of the different root regions. This method is restricted to substantial roots of beans etc. but

of this process ought to be carried on under conditions which secure constancy of all processes in the root which do not participate in accumulation, i. e. the »fundamental» processes. The seedlings must be grown under constant light, temperature and carbon dioxide conditions. In the experiments also temperature, air supply etc. ought to be kept constant.

The inevitable individual differences can be eliminated in two ways: Either a large number of similar plants is divided up into groups of 15 to 20 individuals, and these groups are simultaneously exposed to varied salt conditions, or each group is treated as an individual and successively subjected to varied salt conditions. Both these methods were employed in the experiments. The absorption and the metabolic activity, coupled with it, will then appear as a corresponding increase (or decrease) in the fundamental metabolism of the root system. Experience confirms the exactitude of these methods. The total salt absorption is measured by differential analysis of the solution before and after the experiment or by ash analysis. The final result of the transport is determined by analysis of the bleeding sap, if cut roots are used. Submerged roots or parts of roots render possible only the determination of the part of salts which remains transitorily accumulated in the root tissue.

The general method of studying the salt uptake when using a multicellular absorption organ is identical with several other plant physiological methods, e. g. for studying the carbohydrate assimilation. Whether leaves or unicellar algae are used makes little difference, if only the fundamental conditions are kept constant (including the control of stomate movements in the leaves). It is impossible to distinguish the reduction of CO_2 from the simultaneously proceeding respiration. It is also impossible to distinguish the production of glucose from the transport of glucose within the cell or from

is not applicable to thin grass roots. Determinations of the absorption and respiration of small zones of single roots are of course more exposed to error than are studies of intact root systems.

one cell to another within the leaf. Nevertheless careful experiments have revealed many biochemical characteristics of the assimilation process.

Summarizing: For the study of the mechanism of salt absorption it is important to use absorption organs, such as roots, just as studies in assimilation ought to be performed on assimilation organs. When using roots the experiments show the totality of absorption, accumulation and transport, but it is possible to divide up this complex to a certain extent.

3. Absorption, accumulation, translocation.

Theoretically the total complex of the uptake of salts can be divided up in three elementary processes: 1. the absorption in the boundary between solution and protoplasm, 2. the accumulation within cells or organs, and 3. the transport to other cells or organs.

In colloidal chemistry absorption means usually the exchange of ions at the boundary between medium and colloid particles. In the preceding chapter, supported by previous potential measurements, we arrived at the conclusion that the surface of the protoplasmic membrane is probably built up as a monolayer. Some of the molecules extend their electronegative valencies in the solution and keep cations from it bound, »absorbed». The amphoteric character of several lipoids, e. g. phosphatides makes it very probable that also positive valencies occur in the membrane and that the latter is in reality covered by a mosaic of positive and negative valencies, which are saturated by cations and anions respectively from the bulk phase. The existence of oriented molecules in the membrane prevents a mutual elimination of the opposite valencies (intermolecular bindings). The molecular forces cause a polar orientation of the molecules and a certain compression in the monolayer (ADAM 1930, LANGMUIR 1939, DANIELLI 1937, TEORELL

1939), which probably is one of the causes of restrictions of the free movement of water and dissolved substances through the protoplasmic membrane.

When the protoplasmic membrane is in contact with a very diluted acid the negative valencies are expected to be saturated with H-ions and the positive valencies with OH-, or to a small degree with HCO₃-ions. If a neutral salt is added to the water, partial exchange commences: the H-ions at the membrane are exchanged for metallic cations and the OH ions are also probably exchanged for the anions of the salt. The cation exchange can be demonstrated by changes in the potential. For the existence of an anion exchange further evidence will be given later.

This conception of the absorption phase of salts is supported by a large number of investigations in this laboratory in the course of more than ten years.

The exchange of metallic cations has been demonstrated by the chemical analysis of attracted and repulsed ions. This exchange obeys well-known laws of colloidal chemistry (diameter, charge, hydration of ions). A number of series, published in 1932, demonstrate these rules in the homologous series of alkali and earth alkali metals. From the chlorides of these metals the absorption of cations and output of Na and Ca, as well as the absorption of Cl, was determined. Seedlings of wheat and peas were used. The duration of experiments for wheat was 24 h. See table 3.

These experiments show that plants, grown in water with Na but little Ca, after transfer to chloride solutions of other cations give off much Na but little Ca, whereas plants grown in pure Ca solutions under similar circumstances give off less Na but more Ca. The latter plants have apparently lost much Na in the Ca solution and therefore hold more Ca than Na absorbed. That the output of Na and Ca ions is the result of an exchange with the cations in the chloride solution is shown by the fact that the amount is dependent of the amount of cations taken up, whereas in conformity with

Table 3.

From LUNDEGÅRDH, BURSTRÖM & RENNERFELT 1932.

All values in milliequivalents.

Salt 0.0025 n	Cations absorbed	Anions absorbed	Na given off	Ca given off	Quotient $\frac{M^+}{A^-}$ absorb.
A. Wheat seedlings, grown in tap water (contain but little Ca)					
LiCl	0.520	0.460	0.134	trace	1.13
NaCl	0.040	0.160	—	0.044	0.25
KCl	0.960	0.700	0.476	0.052	1.37
RbCl	0.720	0.920	0.576	0.076	0.78
CsCl	0.620	0.080	0.434	0.064	7.75
B. Wheat seedlings, grown in 0.0025 n $\text{Ca}(\text{NO}_3)_2$.					
LiCl	0.320	0.200	0.008	trace	1.60
NaCl	0.160	0.440	—	0.076	0.36
KCl	0.900	0.460	0.070	0.108	1.96
RbCl	0.520	0.540	0.078	0.092	0.96
CsCl	0.440	0.160	0.058	0.088	2.75

exchange laws for colloids, the larger cations have a comparatively more intensive exchange effect than the smaller ones.

The results described in the publication named have been repeatedly obtained in several later experiments. The output of cations is by no means abnormal, because, after the experiments, the roots continue to grow unaffectedly. It is the normal reaction on unbalanced solutions. In balanced solutions, which contain Ca, this element is not given off, unless, owing to previous overfeeding with Ca, very much of it is present in the organ. The same is the case with all other elements. The conclusion is that the absorption surface of the root is covered by an assortment of cations, which results from the absorption competition during the contact with a solution. Cations which are not represented in the solution

are then most easily exchanged (monovalent ions of course to a larger extent than bivalent ones etc.). If the root has previously been fed with a great deal of one ion, e. g. Ca, this cation can to some extent be given off even in a weak solution of the same ion. Hence the rules of exchange at colloidal surfaces are valid in all details.

4. Root potentials.

The results of the chemical determination of the cation exchange were verified by potential measurements. The general method of determining the surface potential of the root was outlined above (p. 242 and fig. 1 and 2). Comparative experiments in diluted acids, acid salts and neutral salts lead to the important conclusion, theoretically expected, that the measured potential is chiefly a function of the H-ion concentration on the root surface (LUNDEGÅRDH 1938 a, 1939 a). In diluted acids, in the absence of metallic cations, the wheat root showed a cH of approximately 10^{-3} n. If a neutral salt is added, ZcH diminishes, because the H-ions are exchanged for metallic cations. In a solution of a neutral salt of pH = 7 the exchange power of the cations is very prominent. Even in a 0.00001 n solution of KCl approximately 90 % of the H-ions are exchanged at the root surface for K-ions, corresponding to a ZcH of c. 10^{-5} , instead of a maximum ZcH = c. 10^{-3} . Thus the slightest traces of metal ions are absorbed from distilled water. This is probably the explanation of the »oligodynamic» phenomenon, e. g. the poisonous effect of traces of copper.

In acid solutions the exchange power of metallic cations is diminished, and reaches zero at a LpH, which corresponds to the ZpH, i. e. c. 3 for our wheat roots. This fact explains the detrimental effect of low pH-values on the absorption of nutrient cations. The exchange power of course increases again with the concentration

of the salt, because the quotient $\frac{M^+}{H^+}$ in the solution decides the direction and magnitude of the exchange. Earlier physiological experiments, as well as practical observations in agriculture, pointed in the direction that the growth retarding effect of low pH-values can be eliminated by adding salts of, e.g. calcium to the substrate. I venture to suggest that the balance $\frac{M^+}{H^+}$ in the root surface is one of the deciding factors.

To sum up: The surface potential of the root approximately conforms to the formula $-E = \frac{RT}{nF} \ln \frac{ZcH}{LcH}$ or, at 20° centigrade $-Emv = 58 \cdot \log \frac{ZcH}{LcH}$. Here is $ZcH = cH$ at the root side of the boundary, $LcH = cH$ at the solution side. If, in wheat roots, $ZcH = 10^{-3}$ the potential difference will be zero at $LcH = 10^{-3}$. At $LcH > ZcH$ the protoplasmic membrane of the roots will be charged positively against the solution: this is an abnormal situation, which very soon leads to the disorganization of the membrane and death. The destruction of the membrane begins with an abnormal rise in the ZcH -value. At $LcH < ZcH$ the membrane is charged negatively in comparison to the solution: this seems to be the normal state of the root.

Surface potentials of roots have been previously measured by several investigators. In these earlier observations, unfortunately, the fundamental influence of the O-boundary potential and the ion exchange in this boundary have been neglected. The importance of this question necessitates a full elucidation of this point.

A living cell surface develops no static electricity. The only kind of possible charge is therefore an electromotive power developed in an electrical double layer. The root surface — and generally all cell surfaces — are saturated with water: an electrical double layer always exists in the boundary between the protoplasm and the cell wall. The origin of this

double layer is the fact that the charged surface of the protoplasm tends to attract ions of opposite sign to it. The ion activity of the protoplasmic membrane is probably due to its own ionization.

According to the theory of NERNST the source of potential in the boundary is the tendency of the ions to move in the direction of the diffusion gradient, thus from the protoplasmic membrane to the water film in the cellulose membrane, and the height of the potential depends on this gradient $\left(\frac{c_z}{c_1}, \right.$ if c_z is the ion activity in the protoplasmic membrane and c_1 is the activity in the cell wall) and on the relative ion mobility. Because of the fact that the mobility of the H-ions is very much larger than that of any other ions, the relation $\frac{ZcH}{LcH}$ will be the dominating one (see above). If $ZcH > LcH$ the protoplasmic membrane becomes electro-negative, because the positively charged H-ions have a tendency to move into the cell wall and thus diminish the positive charge of the membrane. In consequence the wall will become electro-positive to the same degree. If we apply an electrode to the cell wall, we therefore measure the charge of the cell wall and not of the protoplasmic membrane. This is the reason why some earlier investigators speak of a positive charge of the root surface. What they have measured is the positive charge of the water film in the cell wall, whereas the protoplasmic membrane itself carries an equal negative charge.

A further important conclusion can be drawn from the theoretical analysis of the problem. Because of the fact that the potential of the protoplasmic surface is always the potential of an aqueous electrode, it has no absolute value. It is always bound to the relation $\frac{ZcH}{LcH}$. A measurement of the potential is therefore meaningless unless the value LcH is known, the

more so as normally roots are always surrounded by a solution. The solution can be regarded as a continuation of the moist cellulose membrane. No facts indicate that the cellulose membrane would itself create any boundary potential at its outside, because the native cellulose is not dissociated to any measurable degree. Observations on dead roots show no potential differences. If, therefore, an electrode is placed in the solution in which the roots grow, the potential measured is due to the outside of the electrical double layer in the boundary between protoplasm and cell wall. And the potential of the inside of the layer, the surface of the protoplasmic membrane, is the same, but with reversed sign. Earlier investigators as a rule made the mistake of identifying the charge of the solution with that of the protoplasmic surface.

The cell potential must be measured by means of a non-polarizable liquid electrode. A metallic electrode in contact with the moist cellulose membrane receives a charge (positive for precious metals, negative for non-precious metals) which deranges the measurements. A general form of liquid electrodes is a weak salt solution, which, by means of a tube filled by KCl-agar, is connected with a Zn-Hg-ZnSO₄-electrode. Earlier investigators generally disregard or ignore the importance of a definite composition of the liquid. Some use, e. g. »tap water», thus a mixture of salts of unknown and perhaps varying composition. A glance at figures 3 a. 5, which show the change in potential when a pure acid or a single salt is used in varying concentrations, reveals at once the uselessness of a potential measurement of the biologic potentials, unless the liquid electrode is exactly defined.

The existence of diffusion potentials in living cells and tissues must not be overlooked. Such potentials arise, if a membrane separates two electrolytes of different concentrations or different compositions. Generally the source of diffusion potentials is the different mobility of the ions in a membrane. Several physiological chemists have constructed models in which diffusion potentials induce accumulation of cations

(TEORELL 1933, 1937, MEYER 1937), but it has never been proved that such models can explain accumulation of salts in living cells. OSTERHOUT (1936) describes a model in which a diffusion potential is induced by the diffusion of carbon dioxide through a membrane. This artificial »cell» is capable of accumulating potassium — or any other cation — but not anions. Besides that, very little is known about the diffusion potential of a weak acid such as H_2CO_3 , especially in the case where cH is very different on both sides of the membrane, as is the case in respect of living cells. In our case diffusion potentials must be excluded as the possible explanation of root potentials (LUNDEGÅRDH 1938 a). On p. 246 I showed that the anions of acids do not interfere with the potentials. If diffusion potentials determined the observed potentials, the migration velocity of anions, as well as of cations, would have a marked influence. I also call attention to the fact that no potential arises if the root is exposed to different salt concentrations at low LpH -values. When $\text{LcH} = \text{ZcH}$ the potential is zero. If, then, a salt were added to the solution, a diffusion potential would arise, if such potentials play any role in the absorption problem at all. But the potential is still unaffected. This experiment proves that the source of the root potentials is the H -ion charge in the membrane and not diffusion potentials, and the change of potential observed at »normal» LcH -values is due to exchange between metallic cations and the H -ions at the membrane, as described above.

The fact, mentioned here and in previous papers (LUNDEGÅRDH 1938 a, 1939 a), that a regular diffusion potential is not developed at the surface of the root, even with very steep gradients of salts, seems to support the assumption that a regular diffusion of salts through the protoplasmic membrane does not normally exist. I recall again the discussion on p. 237, in connection with OSTERHOUT's statement that the protoplasmic membrane exhibits a very high electrical resistance. The passage of ions moved by »diffusion forces» is apparently very

limited. We have explained this fact by the assumption of a membrane of oriented molecules. If metallic ions and anions of strong acids are »adsorbed» or chemically »bound» at the outside of the membrane and, by the turning over of the molecules, carried to the inside of the membrane and detached here by some chemical process, this theory would explain why diffusion potentials in the classical meaning will have little chance of being developed. It seems to me that OSTERHOFF's ideas as to a temporary binding of the salt ions in the membrane are supported by my results on the non-occurrence of a diffusion potential.

If the phase boundary potential at the surface of the cell depends on ions — primarily cations — loosely anchored to the surface of the protoplasmic membrane, the thermodynamical principles of a membrane equilibrium will probably nevertheless be valid. The cation exchange at the membrane is regulated by this equilibrium (see equation 1, p. 241) and from this point of view the potential is only an expression of the ion balance brought about between a primarily charged membrane and a dissociated solution.

The importance of biological potentials is twofold. Firstly a potential, measured under normal biological conditions, — e. g. the root potential in a normal nutrient solution or the potential of kidney epithelium in normal urine — demonstrates the electromotive forces which participate in the normal life of the cells and tissues. These forces are capable of regulating the ion exchange, because ions of similar charge are retarded, ions of opposite charge accelerated. If, in some way, a circuit is closed inside or outside the tissue, they also largely influence the transport of charged particles (= electro-cataphoresis and electro-endosmosis). Secondly, the magnitude and sign of the potential renders possible a calculation of the H-ion activity in the boundary, which contributes to the knowledge of the protoplasmic membrane, as demonstrated above.

5. Adsorption of cations. Stability of the protoplasmic membrane.

If cations are adsorbed in the membrane by means of an exchange with H-ions, the adsorption capacity will be decided by the maximum of H-ions held by the membrane. This maximum is attained if the cell is treated with a diluted strong acid ($< 10^{-3}$ n). The symbol is ZcH_{\max} . This value can be determined from the potential in a diluted acid, according to the formula

$$-E_0 = 0.198 T \cdot \log \frac{ZcH_{\max}}{c. \text{ acid}}, \quad (4)$$

if $c. \text{ acid}$ is the concentration of the diluted acid during the determination. E_0 is here mv.

As stated above, an exchange of the H-ions in the membrane with metallic cations will immediately begin, if the cell is transferred to a solution of a neutral salt. The extent of the exchange is reflected in the falling value of $-E$. If ZcM metallic cations are absorbed by exchange against the same quantity H-ions in the membrane the quantity ZcH_{\max} must be reduced by ZcM to give the actual ZcH_1 value. The potential will then be determined by the equation

$$-E_1 = 0.198 T \cdot \log \frac{ZcH_{\max} - ZcM}{10^{-7}} = 0.198 T \cdot \log \frac{ZcH_1}{10^{-7}}. \quad (5)$$

Here 10^{-7} is the concentration of H-ions in the neutral solution of the salt.

The relation between the concentration of the salt ($= LcM$) in the solution and the potential of the interphase of the root is very simple and conforms completely to common adsorption rules (see fig. 5), according to the equation (cf. LUNDEGÅRDH 1939, p. 170)

$$\log (ZcH_{\max} - ZcM) = \log (ZcH_1) = -a \log (LcM) + \log K. \quad (6)$$

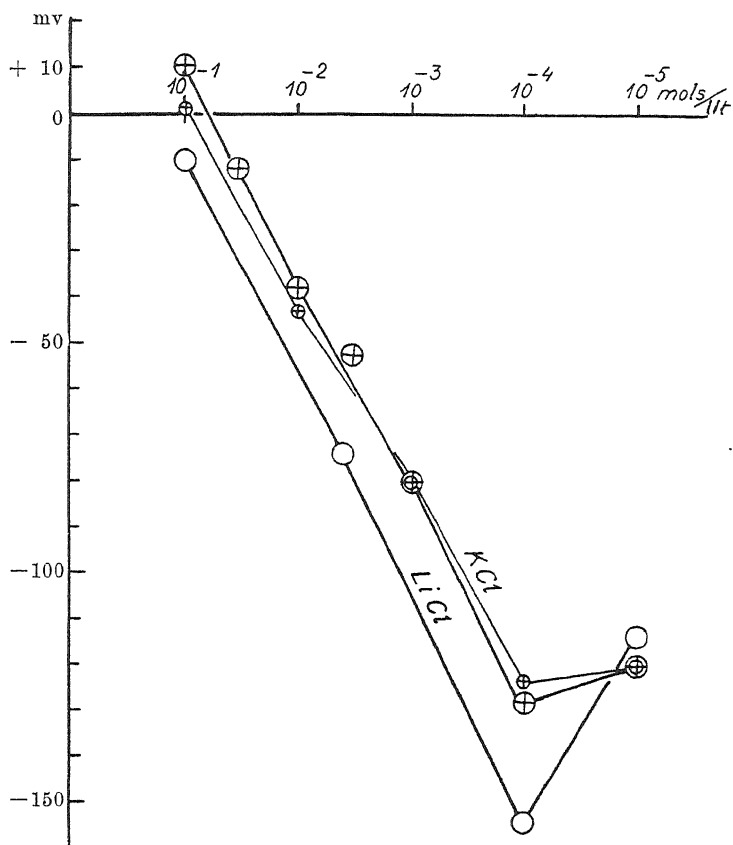


Fig. 5. The relation between surface potential of the root and the salt concentration (on the abscissa) in the solution. Two parallel series of KCl and one series of LiCl.

According to formula (5), $\log (ZcH_1)$ is proportional to $-E_1$ which is a general statement of the fact, visualized in fig. 5, that the potential varies inversely with the concentration of the cations in the solution. This statement accords with a number of experiments by other scientists on animal tissues, e. g. muscle fibres (FRANCIS 1937). The phenomenon is usually (cf. HÖBER 1926 p. 732) explained as a result of diffusion

potentials, induced by the salt (e.g. KCl). I have demonstrated in the foregoing that the hypothesis of diffusion potentials does not hold good for our roots. The theory of a charged membrane and adsorption of cations by exchange against H-ions in the membrane affords a satisfactory explanation of all observed facts.

In earlier series of chlorides of Li, Na, K, Ca and Ba the calculated exchange power increased decidedly in a similar manner to that of the adsorption power of cations in colloids (LUNDEGÅRDH 1938 a). Thus K exchanged more H-ions than Li, Ba more than Ca, etc. In equinormal solutions Li-salts give a lower negative potential than K-salts (fig. 5). This fact is important in view of the absorption mechanism of salts (see below). A series of new experiments regarding the potentials in Li- and K-chlorides is quoted in table 4.

Table 4.

Potentials, 2 minutes after start, on the lower 20 mm of isolated roots of wheat.

Concentration	0.00001 n	0.0001 n	0.001 n	0.0033 n	0.01 n	0.033 n	0.1 n
1. KCl	-120 mv	—	-76 mv	-52 mv	-31 mv	-12 mv	+11 mv
2. LiCl	-145	-118	-84	—	-45	—	-8

PALLMANN (1938) advances a rule according to which the exchange power of alkali-cations increases in proportion to the ionic diameters in Å (cf. also ZADMARD 1939). He refers to experiments with humus colloids. I have earlier (LUNDEGÅRDH 1932) mentioned the fact — supported by a large number of observations — that the behaviour of the protoplasm to inorganic ions reflects in all details the behaviour of amphoteric colloids in general. Recently BURSTRÖM advanced a number of interesting observations on the exchange of cations in the protoplasm: Mn-ions, which in the adsorbed state are normal constituents of the protoplasm

(see below), are exchanged, in solutions of single salts, for other metallic cations and this exchange obeys general laws of exchange in colloids. Mn has a specific catalytical effect upon the nitrate reduction in roots, so that the very small amount of adsorbed Mn could be traced by its influence on this process.

The formula (6) is approximately correct for values of LcM between 10^{-1} and $10^{-4}n$, a fact which indicates a high stability of the protoplasmic membrane in the corresponding region of ZcH_1 values. These values can be calculated from equation (5) and are c. 10^{-5} to c. 10^{-7} . As, in neutral solutions, the LcH value is 10^{-7} , we find that the membrane is stable within the relations of $\frac{ZcH}{LcH}$ from c. 100 down to c. 1.

In experiments with diluted acids we found a stable region between more or less the same limits (see p. 246): the high limit was reached at $LcH = c. 10^{-5}$ giving $\frac{ZcH_{max}}{10^{-5}} = \frac{10^{-3}}{10^{-5}} = 100$, and the low limit at $\frac{ZcH_{max}}{LcH} = \frac{10^{-3}}{10^{-3}} = 1$. In experiments with neutral salts LcM -values above c. 10^{-1} give too high ZcH values: the conclusion is that the membrane holds more H ions than calculated, i. e. the membrane is abnormally acid. Just the same thing was observed for pure acids of $cH > 10^{-3}$ and our conclusion that the membrane becomes unstable when it is discharged $\left(\text{at } \frac{ZcH}{LcH} = 1 \right)$ is thus confirmed.

At values $\frac{ZcH}{LcH} > 100$ the observed values of ZcH are too low: the membrane in this case holds too few H-ions. An example of this fact is afforded by fig. 5. The potential curves show a sudden drop at 10^{-5} m of KCl or LiCl. This change in the activities of the membrane does not seem to imperil the life of the cells, at least at LcH values of c. 10^{-7} , because roots live for a long time in very diluted solutions of single salts. Nevertheless the decrease of the H-ion activity of the

membrane must diminish its absorption power. A very interesting confirmation of this conclusion was obtained by BURSTRÖM (1940), who recently found that the cation absorption in diluted solutions shows a minimum at $\text{pH} = 7$. The anion absorption was not affected by the pH -value of the solution. The minimum is rather sharp, because the absorption rises from zero at $\text{pH} = \text{c. } 3$ to considerable values at $\text{pH} = 6$, quite in accord with our calculations from the potential measurements.

6. The interference of anions in the O-boundary potential. The possible chemical nature of the negative valencies in the membrane.

We learned from potential measurements in different solutions that the principle of diffusion potentials (see equation 3) is not applicable to the root surface. The potential is created by a stable charge in the membrane, caused by a number of negative potentials, which hold H-ions or, in salt solutions, partly H-ions and partly M-ions «adsorbed». The measured potential is an expression for the concentration of H-ions per unit surface of the membrane. A balance exists between H-ions and M-ions similar to the thermo-dynamical equilibrium expressed in equation (1).¹

Our rejection of the principle of diffusion potentials was based on the experiment, among others, showing the lack of influence of the anions of strong acids (HCl , H_2SO_4 , HNO_3 , H_3PO_4) on the potential of the root membrane (p. 246). The curve plotted in fig. 3 had almost exactly the same course, if any one of these acids were used in a dilution series. The mean differences in the readings amounted to only one or two millivolts.

¹ This balance can be calculated from the equations (4—6) and appears generally as the fact that LcM and ZcH are inversely proportional to each other.

These results looked somewhat puzzling from the point of view of salt absorption. If diffusion potentials do not exist, this proves also that salts are normally not absorbed by diffusion. For cations the absorption mechanism was revealed as an ion exchange (ion adsorption). For anions, *ceteris paribus*, a similar mechanism was postulated. But if anions are absorbed by exchange, also positive valencies must exist in the surface of the membrane. And these positive valencies ought to be saturated by OH (or perhaps to some degree HCO_3) ions, and these OH-ions must again to some extent influence the potential. It can be assumed *a priori* that the surface concentration of the positive valencies is much lower than that of the negative valencies. If the ZcH -value is high — as is the case if the solution is a diluted acid — the influence of the positive valencies will be very small. This is presumably the reason why the anions did not produce any noticeable effect on the potential. The experiments were, for this reason, repeated with salts of different anions.

Table 4 shows some results of potential measurements in different potassium salts. We notice that the negative potential increases somewhat with anions in the order $\text{SO}_4/2 < \text{Cl} < < \text{NO}_3 < \text{H}_2\text{PO}_4$. This series recalls old series on the adsorption of anions in colloids (FREUNDLICH 1930, p. 293). According to these, they are adsorbed in the order $\text{SO}_4/2 < \text{Cl} < \text{NO}_3$. New experiments by ZADMARD (1939) gave the series $\text{Cl} < < \text{NO}_3 < \text{HPO}_4$. NO_3 is consequently always more strongly adsorbed than Cl etc.

Transferring these physico-chemical results to our case, we must conclude that NO_3 is more intensively exchanged for OH-ions in the membrane than is Cl etc. If, now, the OH-ions influence the potential of the membrane, according to the negative charge of OH, their influence would be an increase in the positive charge, i. e. a decrease in the negative charge. A removal of OH-ions through exchange with anions of an acid would consequently again increase the negative charge. This is just what we observe in table 4: Anions of

higher adsorption power give lower negative membrane potentials than anions with lower adsorption power. If dilution curves for different salts are compared, the curve for nitrate will consequently have a lower course than the curve for Cl etc. This was observed already in my paper of 1938 (fig. 7 in that paper).

Table 4.

A. Potentials of the lower 20 mm of isolated roots, 2 minutes after start of experiment.

Concentration	0.001 n	0.01 n
KCl	— 31 mv	— 76 mv
KNO ₃	— 49 "	— 98 "

B. Potentials of the lower 30 mm of roots of intact plants. Measurements 60 minutes after start.

K ₂ SO ₄ /2	— 31 mv
KCl	— 35 "
KNO ₃	— 43 "
K-phosphate (pH = 7)	— 49 "

Our conclusion that anions are also absorbed in the protoplasmic membrane by means of an ion exchange, A⁻ for OH⁻,¹ actualizes the question why the accumulation of anions is so little influenced by the pH value of the solution, whereas, according to investigations of BURSTRÖM (1940) and BURSTRÖM & BORATYŃSKI (1936) the cation absorption is markedly affected. This is no doubt due to the fact that cations and anions are absorbed by means of different mechanisms. We mentioned previously experiments which demonstrate the high degree of independance of cation and anion accumulation. The points in the surface of the protoplasmic membrane which bind cations, are apparently separated from the points which attract anions. We have pictured

¹ In all potential experiments the solution was continuously renewed. The pH of the solution is therefore not influenced by the H- or OH-ions which are thrown out from the absorbing surface.

the membrane as a mosaic of positive and negative valencies. It seems most feasible to assume that the negative groups are an acid and the positive ones are ammonium bases. The phosphatides carry both of these groups, but it seems to be less probable that one single molecule should be able to bind simultaneously one anion and one cation. If different molecules of one kind or of different constitutions bind one category of ions, this would render possible such an individual absorption as the observed facts seem to indicate.

From the ZcH values it is possible to calculate the mean mutual distance of the negative valencies, on the presumption that activities are set approximately equal to concentrations. If $ZcH_{\max} = 10^{-3}$, the number of H ions per square cm is $1/1000 \cdot 6.06 \cdot 10^{23}$. The mutual distance of the H ions will be

$$\frac{1}{\sqrt[3]{6.06 \cdot 10^{20}}} = 0.118 \cdot 10^{-5} \text{ cm} = 118 \text{ \AA} \text{ units. This is}$$

of course an average, because we do not know anything about the uniformity of the distances. From observations on monomolecular layers the mutual distance of the molecules of fatty acids or phosphatides in such a layer can be calculated. It is c. 7—9 Å (ALEXANDER & TEORELL 1939). The negative valencies which constitute the cation adsorption power of the protoplasmic membrane can accordingly control only a minor part of the organic molecules in the protoplasmic membrane, even if these are larger than the molecules of phosphatides and similar substances. A molecule of oleic acid covers an area of c. 46 Å², and a molecule of tripalmitine 63 Å². A molecule of lecithine covers an area of 70—100 Å². If there is only one active H-ion on an area of 118² = c. 13,800 Å² a large number of neutral molecules will apparently separate these H-ion »islands». The number of exchangeable OH-ions is probably much lower than the number of H-ions per unit area, but our calculation shows that there will be plenty of space for both kinds of valencies in a membrane built up

as a mosaic of large molecules. From this point of view there are no difficulties in the way of understanding the individual behaviour of the cation and anion exchange.

The kinetical aspects of monomolecular layers are no doubt different from the theory of uniform solutions. The molecules in a monomolecular layer have a restricted mobility and their behaviour seems to be intermediate between that of a crystal and that of a solution. For the calculation of their activity in a solution one postulates the free mobility of the particles. The activity coefficients calculated for solutions cannot therefore be employed for the surface of the protoplasm. The following calculations, therefore, can only be regarded as rough approximations accepted for activity = concentration and the assumption that molecules in a monomolecular surface behave as molecules in an ideal cross-section of a solution.

The first dissociation constant of lecithin and other phosphatides is $pK_1 = c. 1$ (CHAIN & KEMP 1934). The phosphoric acid group of lecithin is consequently dissociated to c. 99 % at $pH = 3$ and to c. 99.999 % at $pH = 6$, thus practically completely dissociated for all sustainable pH -values (see above). A protoplasmic membrane which is built up of pure phosphatides would consequently carry one H-ion on each c. 9 Å; thus, calculated by volume, corresponding to a $\left(\frac{11.8}{9}\right)^3 = c. 2.25$ molar solution. Potential measurements, as well as other observations, show, on the contrary, that the H-ion concentration of the protoplasmic membrane corresponds to c. 0.001 mols/liter. If phosphatides are present in the membrane, the phosphoric acid group would be directed towards the outside. As mentioned above, the phosphatide molecules must consequently be assumed to form isolated islands in a more neutral membrane substance.

Apart from the conceptions of the composition of the protoplasmic membrane, which emerged from the earlier permeability experiments of several investigators, our recent determinations of the apparent constancy of the H-ion

concentration of the membrane for various LpH -values speak in favour of the view that substances of high acid dissociation, similarly to phosphatides, take part as constituents of the membrane.

The practically complete constancy in the ZcH -value, if the LcH -values were varied from c. 10^{-5} to c. 10^{-3} n, seemed to indicate (LUNDEGÅRDH 1939 a) that the H -ions did not originate from dissociation in the membrane. The new facts about the strong dissociation of the phosphatides make it however possible to explain the observed constancy of the H -ion potential of the protoplasmic membrane from general principles of dissociation.

Isolated phosphatides are to some extent spontaneously oxidized, during which decomposition fatty acids, but not choline, are liberated. The dissociation constant of fatty acids is estimated as about 10^{-5} . This comparatively low value makes it very improbable that fatty acids to any appreciable degree compose the protoplasmic membrane. At $pH = 3$ the fatty acids are dissociated to c. 1 %, at $pH = 4$ to c. 10 %, at $pH = 5$ to c. 50 % and at $pH = 6$ to c. 90 %. If the main substance of the membrane consisted of fatty acids, this would consequently involve a tremendous variation in the ZcH -value in the region $LcH = 10^{-6}$ to 10^{-3} n, for which on the contrary a high stability of the ZcH -value was observed. It must therefore be concluded that fatty acids, if they are present at all in the protoplasmic membrane, constitute only a minor part of it, say 10 to 20 % of the assumed number of phosphatide molecules. The following conclusion can be drawn: The majority of the molecules which build up the membrane will no doubt be substances possessing no dissociation power at all. As islands in this indifferent substance, at a linear distance from each other of at least ten times the molecular section, lie molecules of a high acid dissociation power.

Certain earlier observations of HANSTEEN-CRANNER (1922) and LUNDEGÅRDH (1932) speak for the presence of phosphatides in

the protoplasmic membrane. LUNDEGÅRDH, BURSTRÖM and RENNERFELT (1932) mentioned a liberation of organic substances containing phosphorus in unbalanced solutions. Full evidence that these substances really are phosphatides has not yet been obtained. Speculations as to the nature of the main substance of the membrane are of but little use. The suggestion might perhaps be advanced that the protoplasmic membrane contains a large amount of substances which are chemically transformed into cellulose. This will probably be the case in still growing, or in any case young, cells, i. e. the absorption cells of the root (root hairs). Plasmolyse experiments show that the membrane is intimately connected with the cellulose membrane. The formation of cellulose from glucose postulates a chemical mechanism, and it is probable that this mechanism, or part of it, is situated in the membrane. According to this hypothesis, the main substance of the protoplasmic membrane of young vegetable cells could be characterized as »procellulose». In older cells, in which also the protoplasm separates without difficulty from the cell wall in plasmolysis, the membrane substance might have a different constitution. The same would be the case for cells of fungi, which are coated by a chitin wall. From this view-point it is interesting to notice that the permeability of *Beggiatoa* (SCHÖNFELDER 1930) shows differences from, e. g., the permeability of *Chara* (COLLANDER 1937 a) and that different kinds of mature cells of higher plants might behave differently in respect of permeability. There is, thus, some evidence in favour of the hypothesis that the main substance of the protoplasmic membrane is in some way chemically related to the wall substances.

If we try to characterize the activities of the protoplasmic membrane more in detail, due attention must be paid to the fact that the presence of OH-ions (base dissociation) has a minor effect on the boundary potential, which is shown to be chiefly affected by the H-ion concentration. If phosphatides cause the acid reaction of the membrane, and if these behave as freshly prepared phosphatides in pure monomolecular

layers, the strong basic dissociation would practically neutralize the effect of the H-ions (the i. e. p. is assumed to be $\text{pH} = 7.5$; measured values c. 6.5, see FISCHGOLD & CHAIN 1934, CHAIN & KEMP 1934), because in the pH region 3—6 (see above) on the positive as well as on the negative side a practically complete dissociation would occur. *We must therefore conclude that the basic dissociation in the membrane is much smaller than the acid one.* The observations on the effect of different anions on the potential (p. 269) point in the same direction. On the assumption that phosphatides cause the pronounced acid reaction of the protoplasmic membrane, the conclusion must be drawn that the basic group is counterbalanced by means of binding to other acid groups within the membrane. The nature of these is quite hypothetical.

Our assumption that *phosphoric acid groups* are carriers of the negative valencies in the protoplasmic membrane is supported by the fact that the charge is to a large extent dominated by the *carbohydrate metabolism*.¹ As is well known, phosphoric acid plays an important role in the first stage of the break-down of the glucose molecule. An intensification of the respiration would, from this point of view, require more phosphoric acid groups, which would lead to an impoverishment of them in the membrane. *Intensified respiration would consequently tend to diminish the negative charge of the membrane. This is actually the case*, as several experiments, which are described more in detail in the following, show.

Conclusions: The electro-chemical behaviour of the protoplasmic membrane indicates the presence of molecules of a high acid dissociation constant. If these molecules occupy areas of 50—100 Å², their concentration in the surface must

¹ After the completion of the manuscript there appeared a notice of CONVEY & BOYLE (1939), which deals with »a mechanism for the concentrating of potassium by cells, with experimental verification for muscle». The authors advance the assumption that »phosphorylated compounds important for the carbohydrate cycle» play a rôle in the cation absorption of muscle fibres.

be somewhat less than 1 %. Fatty acids, if present at all, are presumably represented in the membrane only to a minor degree (less than 0.2 %). The chief part of the membrane surface is no doubt occupied by non-dissociated compounds. If the acid compounds in the membrane are identical with phosphatides, the basic group of these must be almost completely neutralized by some hypothetical acid groups in the main substance of the membrane. Under all circumstances *the basic dissociation in the membrane must be lower than the acid dissociation.*

7. Local potential differences on the surface of the root.

The geo-electrical response.

The potentials of the surface of the wheat roots, which are the back-ground for our discussion of the characteristics of the protoplasmic membrane, refer to the lower ends of roots, which were held in the normal vertical position. According to the research work of BRAUNER (1927) the potential of stems and roots of plants is instantaneously changed, if the organs are changed in their position. BRAUNER speaks of a »geoelectrical» response. This response also occurs in the cut roots which I have used in my experiments. BRAUNER explains the geoelectrical reaction by the differential effect of gravitation on the distribution of cations and anions in the phase boundaries, and it seems to me that no serious objection can be raised to this general conception. Not only inorganic ions, but also the large molecules which constitute the membranes will of course react upon gravity, but it is to be assumed that the dislocation of the small ions will be more prominent. The geoelectrical response which BRAUNER describes is probably the sum of small changes in all phase boundary layers of all cells which build up the organ.

‘ In our case we have tried to isolate the reaction of the boundary between the root surface and the surrounding solution, and our conclusions refer to changes in the charge of

this boundary, if the conditions of all other boundaries are kept constant. We do not know what happens to the root surface, if the root is reversed,¹ so that the tip points upwards. The potential changes suddenly, but it shows a tendency to return to its original value. It looks as if the geoelectrical reaction depended upon a small dislocation of the charged particles in the membranes, which is counterbalanced by a tendency to restore the normal state.

The following experiments show the reaction of the roots to gravitation:

A root of wheat was cut into pieces 15 mm long, which were placed in normal vertical position between two 0.01 n KCl electrodes. The lower end was submerged c. 5 mm in the solution, so that the free part between the electrodes was c. 10 mm. *All the pieces showed a negative potential of the lower end*, larger (19—20 mv) in the two apical pieces, smaller (4—13 mv) in the basal pieces of the root (fig. 12).

This normal reaction of the root lasts for several minutes, and is assumed to demonstrate that the H-ion concentration is normally larger at the lower end of the root than at the upper end.

If similar experiments are performed with roots in the horizontal position, the result is the same: the anatomically apical part of the root is negatively charged as compared with the base. In these experiments the liquid electrodes were glass tubes of c. 1 mm in diameter, which were placed vertically on supports so that the root could be placed as a bridge on two drops, emerging from the tubes (see fig. 6). By moving the supports, which were controlled by micrometer screws, the length of the bridge was varied. A few examples are given below (total length of the isolated root ca. 30 mm):

The basic end of the root rests on the left electrode (see fig. 6), the right electrode is moved from the tip to the base and gives the following readings:

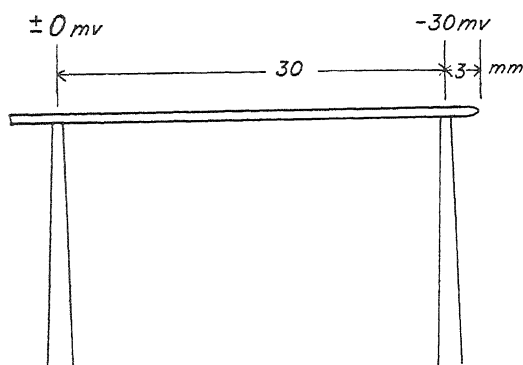


Fig. 6. A scheme of experiments with roots in horizontal position.

	1.	2.
0.5 mm from the tip	— 25 mv	— 22 mv
3 » » » »	— 40 »	— 36 »
6 » » » »	— 45 »	— 10 »
12 » » » »	— 20 »	— 2 »
17 » » » »	—	— 2 »

In several other experiments the normally negative charge of the apical end of the horizontally placed root was confirmed.

If the tip of the root is fixed to one electrode and the other electrode is moved from the base towards the tip, the electromotorical force generally increases with decreasing length of the bridge. We return to this fact below.

Roots which are put vertically, but in inverse direction (tip upwards), show a normal charge in the first few seconds, but in a few minutes this shifts over to a reversed charge. Thus the tip now reacts positively in comparison to the base, whereas the voltage is lower than normally. This experiment shows that the charge of tip and base of the root is partly induced by the gravitation. But the result of the measurements of roots in a horizontal position shows that the ontogenetical differentiation of the organ predominates.

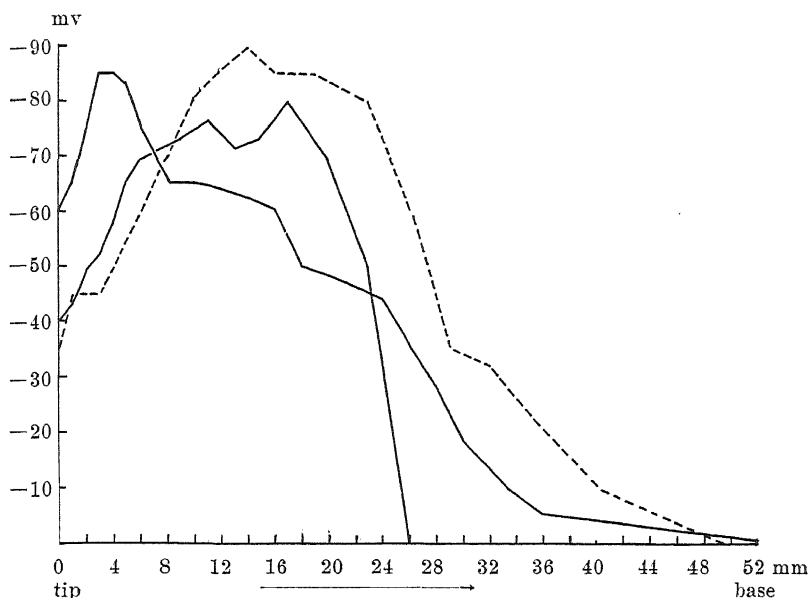


Fig. 7. Potentials in different zones of wheat roots from 3 weeks old seedlings. Upper electrode: 0.01 n KCl. Lower electrode: 0.0005 n CaCl_2 . One of the roots is younger.

If we assume that the geoelectrical response depends upon dislocations of small inorganic ions, this effect will in reversed roots partially overlap the electrical influence of the large organic ions in the membranes, but not cause any enduring structural changes in them.

The charge of the protoplasmic membrane is no doubt a product of the genetical factors. Experiments with different kinds of roots show that, e. g., rye roots have a ZcH value (in diluted acids) of c. 10^{-4} , whereas oats and barley showed approximately the same ZcH -values as wheat.

Ontogenetical differences also occur. Our present investigation covers only roots, and it is to be expected that cells of other organs will show rather deviating charge. As mentioned above, the experimental technique is very difficult for all such determinations, because one always measures two boundary

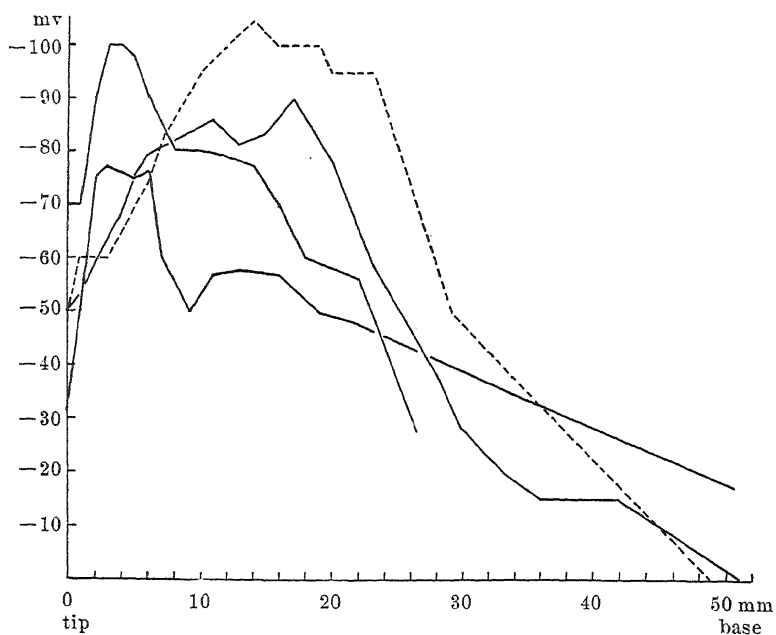


Fig. 8. As in fig. 7. Potentials of four wheat roots, two of the «young» and two of the «older» type.

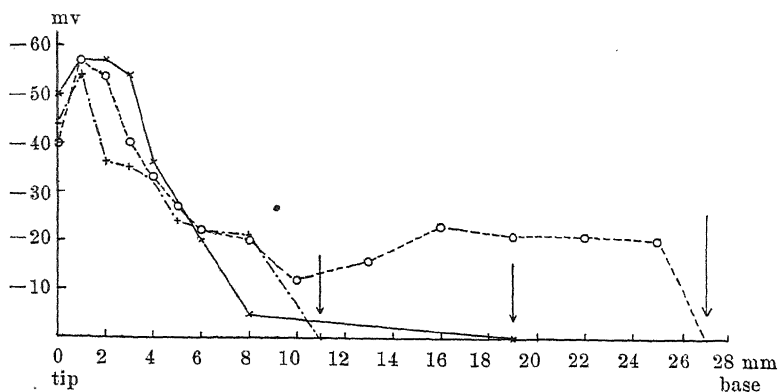


Fig. 9. Potentials in different zones of quite young roots of wheat (seedlings a few days old). Electrode solutions as in fig. 7.

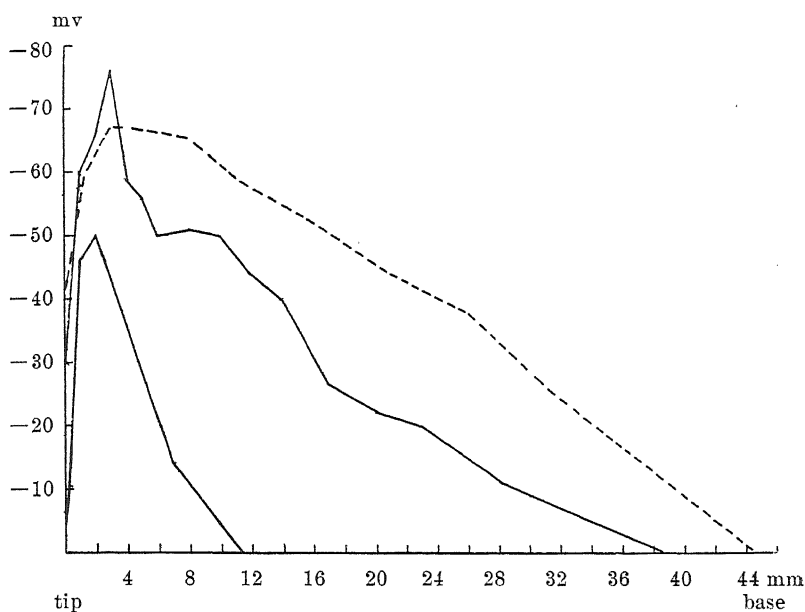


Fig. 10. Potentials in different zones of roots of oat seedlings. Electrode solutions as in fig. 7.

potentials, one at each electrode, and the possibilities of eliminating one of them requires careful study (LUNDEGÅRDH 1938 a). That the ontogenetical differentiation of the root cells involves special behaviour of the surface layer of the root cap, the vegetation point, the zone of elongation, the zone of root hairs etc. can be concluded from the potential at different heights of the root. A number of experiments with roots in a horizontal position were mentioned above.

In view of the importance of a well defined and constant composition of the electrode solution, which is put in contact with the root surface, the following arrangement was chosen for measurements on roots in normal vertical position.

The base of the isolated root was enclosed in the narrow top electrode, which was filled with 0.01 n KCl (see fig. 1). This electrode was carried by a slide, which was moved in

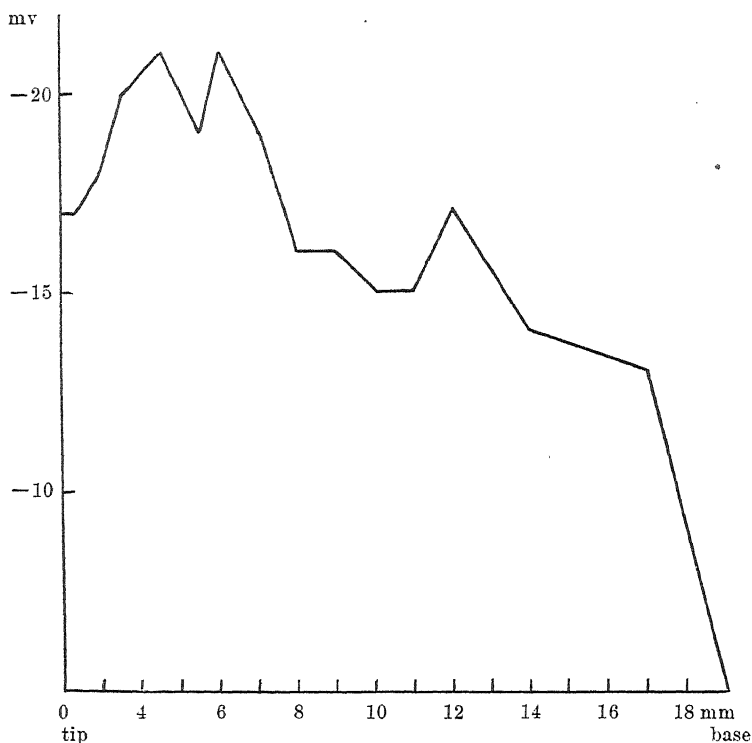


Fig. 11. Potentials in different zones of a pea root. Electrode solutions 0.02 n KCl.

vertical direction by means of a micrometer screw. The lower electrode consisted of an open tube with solution. The upper electrode was gradually lowered, so that first the tip, then more and more of the root was submerged in the lower electrode. The potential was measured at short intervals (see fig. 7—11).

Roots of wheat, oats and peas behave very much in the same way. If only the root cap (c. 0.5 mm) dips into the lower electrode, a potential of c. —30 to —60 mv, relatively, is measured. Further lowering of the root into the solution raises the negative potential up to —90 mv, when 2—4 mm

(in oats and young wheat) or 12—15 mm (in older plants) of the tip is submerged. Then the negative potential decreases again and falls continuously to zero, when the whole root is submerged. Positive potentials were not met with.

The method does not of course render possible an exact determination of the surface potential, because the submerged part behaves as a unit. The striking agreement between these results and the »bridge» measurements described previously (see p. 278 and fig. 6) nevertheless permits of the conclusion that a maximum of negative charge occurs in a zone c. 3—15 mm from the tip, and that from here the charge decreases on both sides.

The interpretation of the unequal distribution of the charges is a rather delicate problem. An important starting point is the fact that the height of the observed minus-potentials of the c. 20 mm long end piece of the roots is within wide limits independent of the total length of the root (see fig. 7—10). This fact leads to the conclusion that the end piece of the roots carries a considerably higher negative charge than the upper basal parts. Observations on roots in horizontal position show that the potential above a c. 20 mm long end piece is sometimes even weakly reversed, if the root is placed as a bridge (see fig. 6). The gentle drop of the minus-potential in the experiments with roots in vertical position (fig. 7—11) is consequently a resultant of a strong negative difference between the tip zone (3—15 mm) and the rest of the root and a low positive or negative potential difference between the base of the root and a medium zone behind the »active» end piece.

The fact that the potential gradient is larger in the end piece than in the rest of the root can also be demonstrated by cutting up a root into pieces and determining the potential differences in each piece. Two such experiments are shown in fig. 12. One of them demonstrates also the geoelectrical effect in reversed roots.

The main results of these observations are the following:

Because of the zonal variations of the potential within the end piece (0—20 mm) of the root the conception ZcH_{\max} has a meaning only as an *approximate average*, which can be used in studying the reactions of the surface. The exact value of ZcH_{\max} must vary within one pH-unit (corresponding to 50—60 mv, see fig. 7—10). The ZcH_{\max} -value of the basal parts of the root is of lower magnitude. Owing to earlier calculations the root base is approximately discharged in 0.01 n KCl (LUNDEGÅRDH 1938 a). Its H-ion activity is consequently c. 10—100 times lower than that of the end piece of the root.

The general behaviour of the different zones of the root, i. e. their reaction to diluted acids, neutral salts etc. is identical (LUNDEGÅRDH 1938 a). We conclude from this fact that the ZcH -value of one zone 3—15 mm above the tip is maximal. A comparison of these results with BURSTRÖM's (1939 b) determination of the zone of absorbing root hairs shows that the ZcH -maximum is attained at a height of the still growing root hairs which coincides with the zone of maximal salt absorption. The ZcH -values on the basis of which we have calculated the activity of the protoplasmic surface refer to the lower 20 mm of the root, and consequently include the zone of maximal absorption power.

The unequal H-ion activity in the root surface induces no doubt weak electric currents, if the current is closed by a conducting solution, which is generally the case under natural conditions. Hence the root tip (3—12 mm) is a micro-element of an electromotive force of 30—50 mv. But the resistance in the thin root is high (a determination gave c. $33 \cdot 10^3$ ohms per mm = c. $3.3 \cdot 10^5$ ohms in a 10 mm long root tip). If the external resistance can be neglected, a current of c. 10^{-7} amperes would arise, with a transport power of c. $4 \cdot 10^{-6}$ milliequivalents per hour. Owing to the negative charge of the zone of elongation (which coincides with the zone of beginning root hairs), anions would be transported from the vegetation point to the zone of elongation of cells, a point of some interest in the problem of auxin transport. Auxin

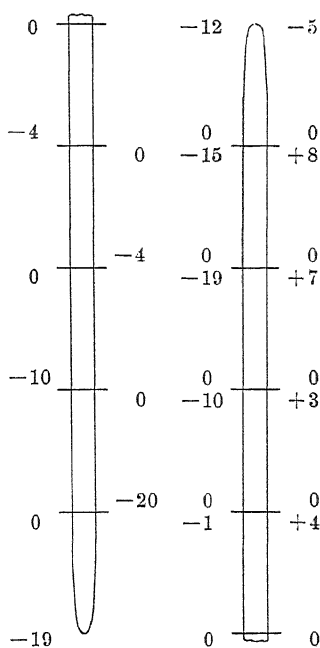


Fig. 12. Potentials in different parts of wheat roots. The roots were cut up into 15 mm long pieces. The potential differences between the ends of each piece were determined in the apparatus fig. 1. Each electrode was filled with 0.01 n KCl. In normal vertical position the potential differences are largest in the 2×15 mm end zone. Also the reversed pieces show immediately after the start similar differences. In 2—3 minutes the geoelectrical effect results in a partial reversal of the potentials (see p. 278). See the figures to the left (0 minutes) and to the right (3 minutes) of the diagram.

is an anion. This question will be dealt with elsewhere (cf. also KINOSHATA 1939, CRAFTS 1939, p. 481).

A reverse current would occur in the upper part of the root, but as the potential gradient here is less steep, the power of transportation would be very small. Our calculation is of course very approximative, but »micro-currents» undoubtedly occur in the roots. As regards the transport of inorganic ions these currents are probably of very little importance. Nevertheless cations and anions of electrolytes will, to some extent, be separated by different parts of the root surface. Thus cations will preferably be attracted to a zone a few millimeters above the tip, just where the root hairs are young, whereas the anions will be more attracted by the somewhat older root hairs. The fact that the anions are »drawn» into the root against the negative potential probably reduces the importance of this weak »electrolysis» brought about by the root end.

Indeed BURSTRÖM (1939 b) was unable to establish any difference in the uptake of K and NO_3 through the root tips. The zone of maximal potential also coincides with the maximum of NO_3 -reduction according to BURSTRÖM's results. Thus *high negative surface potential, high absorption power and high metabolic activity coincide*. A pre-supposition for the endurance of local potential differences on the surface of a root which is surrounded by a conducting medium, is of course the regeneration of the charges. Adsorption alone is an insufficient cause of unceasing currents, for these will tend to create an equilibrium between ion exchange and potential, so that the current ceases. The real source of the enduring potential differences between the root surface and the solution must be sought in the metabolic activity.

The geo-electrical effect, mentioned above, shows that the boundary potential is very sensitive to the relative positions of ions in the cell, which in this case are affected by gravity. Our previous experiments with change of LcH at constant ZcH (in diluted, pure acids) or change of ZcH at constant LcH (in neutral salts of different concentration) showed on the other hand a marked lability of the membrane at extreme values of the relation $\frac{\text{ZcH}}{\text{LcH}}$. We concluded that stability of the

qualities of the membrane postulates values of $\frac{\text{ZcH}}{\text{LcH}}$ between c. 1 and c. 100. This behaviour of the membrane recalls previous investigations in the stability of monomolecular layers of fatty acids, proteins and phosphatides (cf. e. g. RIDEAL 1939). Generally speaking the interfacial tension of such layers diminishes, if the ends of the carbon chains, which emerge into the aqueous phase, are dissociated (DONNAN 1899, PETERS 1931, DANIELLI 1937).

If metallic cations are present in the solution, the protoplasmic membrane eliminates a part of its negative charge through exchange of its H-ions for those. This exchange power increases of course with the potential and thus attains

its maximum in a diluted solution of a neutral salt. This is the reason why every trace of a metal is absorbed from »distilled» water. The so-called oligodynamic effect arises from the maximum negative electric charge in distilled water, which, at a ZcH -value of 10^{-3} , would theoretically be c. $58 \cdot \log \frac{10^{-3}}{10^{-7}} = c. 232$ mv. Even very small amounts of metallic cations in the water reduce the ZcH -value to an endurable degree, but in the complete absence of such possibilities of exchange, the ZcH -value seems to be reduced by a sort of »revolution» within the membrane itself. The nature of this self-regulation is unknown, but it is worthy of mention that a high negative charge of the membrane works thermodynamically in the direction that anions in the membrane tend to be thrown into the solution. In fact earlier experiments show that organic compounds, presumably phosphatides (LUNDEGÅRDH, BURSTRÖM & RENNERFELT 1932, LUNDEGÅRDH 1932), are liberated from the root surface, when the solution is alkalized, i. e. at high potentials. Obviously the organic compounds, carrying a phosphoric acid group and thus behaving as large anions, with the diminished interfacial tension leave the membrane and thus participate in the lowering of the phase boundary potential. The repulsion of anions includes of course not only the constituents of the membrane itself, but also dissolved anions from the inside of the cells. Thus organic acids, and inorganic acids, if present, tend to escape from cells, if the environment is alkalized and the surface potential consequently increases. The output of carbon dioxide is also facilitated by a high boundary potential. We shall return to this problem later.

Our discussion of the characteristics of the protoplasmic membrane has repeatedly emphasized analogies with the behaviour of monomolecular films. I consider that the general view which LANGMUIR (1939) gives as to the biological importance of such films, is well founded. Nevertheless one must bear in mind that the protoplasmic membrane is certainly

built up of more than one kind of molecules, and that it probably has a thickness of many layers of molecules. If chiefly the surface layer determines the charge, ion exchange power etc. and, owing to its compressed structure, holds the single molecules in tight bundles¹, there are again other facts which suggest a metabolic activity close to the surface. The molecules in the surface layer are probably not excluded from this chemical activity of the sublayer or of the bulk of protoplasm. In a pure layer of fatty acids, too, the single molecules are thought to vibrate and from time to time to turn over (LANGMUIR 1939), and the idea of such an interaction between the surface layer and its two faces will assist our further discussion of the absorption and accumulation of ions.

The apprehension of a surface layer of oriented molecules is no obstacle to the general conception of permeability. Owing to the dissociation of some of its molecules and its electrical charge the compression (interfacial tension) of the surface of the protoplasm is certainly not high, and small molecules of a diameter up to 4 Å (LUNDEGÅRDH 1935) will be able to »wriggle» through it. The comparatively low interfacial tension will also allow large molecules (e. g. virus molecules) to sink into the membrane and be devoured by the protoplasm, in a way similar to that in which a plasmodium or an amoeba devours its food. Owing to the high hydrostatical pressure in plant cells, the membrane is intimately in contact with the cellulose wall, especially in meristematal cells. Indicative of this intimate contact are the threads which, under certain circumstances, join the shrinking protoplast in plasmolyzed cells to the wall. The close contact between membrane and cellulose wall might perhaps modify the nature of the interfacial layer or lead to a certain »knottiness».

¹ Artificial monolayers of more than one kind of molecules have also been synthesised (ADAM & JESSOP 1928; SCHULMAN & RIDEAL 1937; RIDEAL 1939).

8. Time course of root potential.

As indicated by our previous studies, the power of the root surface to adsorb metallic cations is closely related to the H-ion concentration of the protoplasmic membrane. This, on the other hand, is probably associated with the acid dissociation of some constituents of the membrane. The adsorption of metallic cations is, therefore, to be compared to the formation of a salt. A striking parallelism to the studies in this laboratory on adsorption, exchange and antagonism of cations is provided by the studies of LANGMUIR and collaborators on the adsorption of cations in monolayers of fatty acids, spread on an aqueous surface. At pH-values which induce a maximum dissociation of the hydrophilic carboxyl-ends of these long molecules — thus preferably on the alkaline side ($\text{pH} > 7$) — cations are intensively adsorbed, even if they are present in very diluted concentration (see above, »oligodynamic phenomena»). Antagonistic phenomena were also observed between sodium and calcium etc. Earlier studies by BURSTRÖM (1934) showed that ion antagonism is a mass competition between ions at the surface of the root. Thus the relation $\frac{cM_1}{cM_2}$ (if M represents a metallic cation) dominates the intensity and direction of the antagonistic action. Generally the cation M_1 expels the cation M_2 from the surface, if $cM_1 > cM_2$. This principle of equivalency has a general bearing also for inorganic colloids. Graphically the absorption of the ion M_1 with increasing amounts of M_2 is visualized by a S-shaped curve which probably has an asymptotical course in the right shank. If cM_1 is much lower than cM_2 , but little M_1 is absorbed, but even with a very large surplus of M_2 , the absorption of M_1 is not completely prevented. This seems to be a very important biological observation, because the phenomenon ensures a minimum absorption of minor elements in a mixture of nutrient cations, especially if the minor element is characterized by a high adsorption capacity (= high valency and

low hydration power). When $\frac{cM_1}{cM_2}$ approaches 1 the antagonistic action increases violently and at the value of 1 turns rapidly over into the reversed state, antagonism of M_1 on M_2 .

The nature of the adsorbed cations largely influence the physical properties of monolayers (LANGMUIR 1939). The surface pressure of the film, which tends to spread the molecules in the interfacial boundary, is caused by the interaction of the dissociated groups and water molecules. If the H-ions are exchanged against metallic cations, the mobility of the hydrophilic ends of the carbon chains is changed. It was frequently found that the binding of potassium in the protoplasm makes it more »liquid», whereas calcium has the reverse effect. In respect of fatty acids in the bulk phase, these differences are exemplified by the different qualities of the soaps. Similar observations of the influence of adsorbed metallic ions on the physical properties were made on soils (JENNY & REITEMEIER 1935; JENNY & OVERSTREET 1938). If the potential of the protoplasmic surface is partly dependent on the surface tension and its influence on the chemical composition of the membrane, the nature of cations adsorbed on this surface will determine the potential.

In a preceding paper it was pointed out that the »exchange potential» of the root surface was established within c. 2 minutes. In this time the cation exchange was completed. In solutions of potassium salts however the exchange potential did not last longer but decreased slowly, until a comparatively low »Arbeitspotential» was reached (fig. 13). Also in pure diluted acids this slope of the potential was observed (fig. 12). Each of the curves in fig. 12 and 13 is an average of 5—6 separate registerings.

In these experiments cut roots, 60—70 mm in length, of wheat seedlings (3 weeks old) were used. The roots were placed in vessels as in fig. 1. As the junction for the upper, cut end of the root was used a 0.01 n KCl solution. The lower electrode, in which the root end was submerged to 20

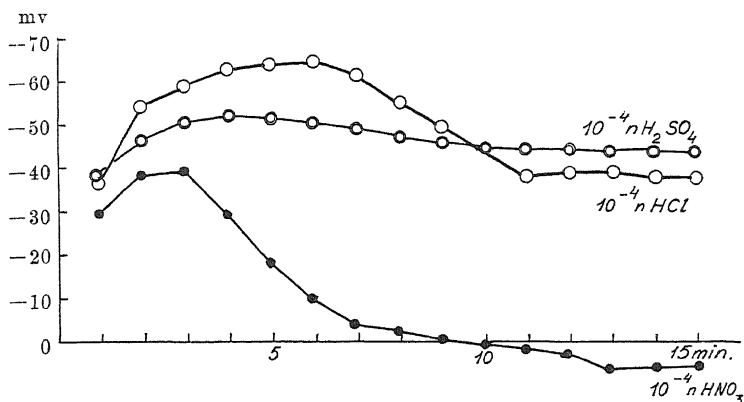


Fig. 12. The time course of root potential in diluted acids. Each curve is an average of 5—6 separate registrations with single roots. These curves are not corrected for the zero-potential of the electrodes. After correction the maxima coincide (see p. 246).

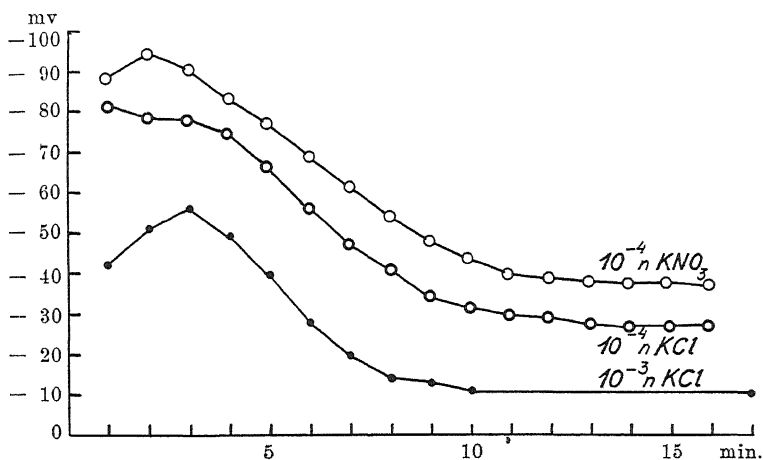


Fig. 13. The time course of root potential in potassium salts as in fig. 12. These curves are corrected.

mm, contained the slowly flowing test solution. The deflections of the galvanometer (c. 10000 ohms resistance) were registered photographically in the usual manner. By means of a comparison with the magnitude of deflection at a known

voltage and the same total resistance (c. 10^5 ohms) the millivolt values were calculated.

The curves show with uniformity the rapid attainment of the optimal exchange potential, somewhat more rapidly in 10^{-4} n than in 10^{-3} n. The maximum of the curves is more flat if acids are used. This perhaps depends on the direction of the exchange. In salts metallic cations move towards the root surface, H-ions in the opposite direction. In pure, diluted acids H-ions move inwards and substitute metallic cations, which were previously adsorbed. Metallic cations pass continuously from the interior of the root (LUNDEGÅRDH 1937, Table XII, XIII, R 40, 46, 51), which might delay the moment of equilibrium.

If a root is transferred from distilled water to a diluted solution of, e. g., KCl the H-ions are chiefly exchanged against K-ions. If the root comes from a complete nutrient solution, the K-ions will exchange plenty of Na, Ca, Mg ions etc. among some H-ions. But it is characteristic of the adsorption in the root surface that the final result will be the same, and the exchange will be completed in about the same time.

If, now, the potential is observed or registered for a longer time (2—3 hours) and such experiments are repeated with different salts of the same concentration, one finds that the fall of the potential is much more striking in salts of potassium than in solutions with Li, Na or Ca. Fig. 14 shows examples of such time-curves. These experiments were performed with seedlings, of which the leaves were cut off at c. 15 mm above the seed. Generally 3 seedlings were held in the potential vessel (cf. fig. 2) by means of a tuft of dry cotton-wool, so that the root ends dipped c. 20—30 mm into the test solution. As the top junction was used a pillar of cotton-wool, moistened with 0.01 n KCl.

In such continuous potential measurements one must take into consideration the fact that to some extent the root creates its own environment. Owing to ion absorption and the emission of carbon dioxide, exactly the root »film», i. e. the neighbouring layer of the solution, gets a composition which differs from

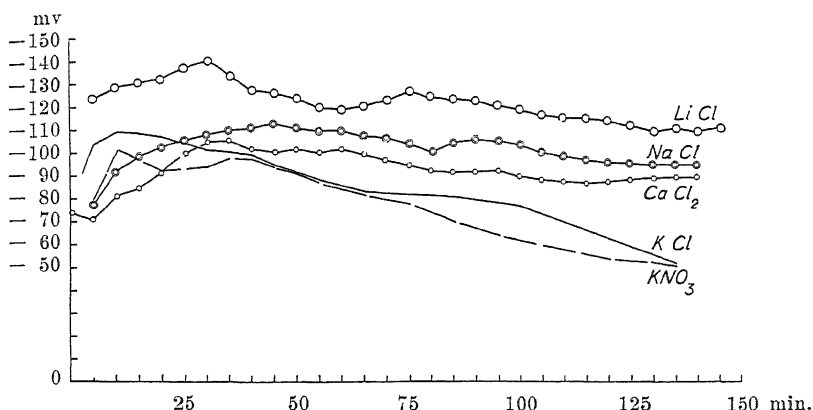


Fig. 14. The time course of root potential. Experiments with decapitated seedlings.

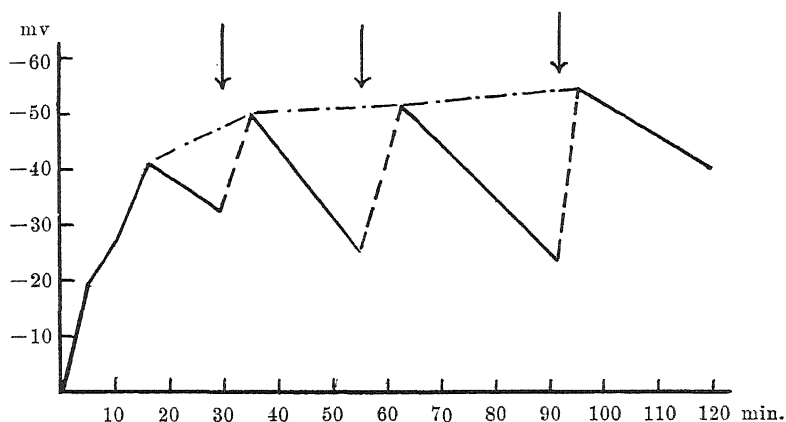


Fig. 15. Potential measurements of a root system, which was held in 10^{-4} n KCl. The arrows indicate the renewal of the solution.

the »free» solution. In a continuously flowing solution, the peculiar behaviour of the root film is less marked. Earlier investigations, e.g. those of NIGHTINGALE (1934), show the importance of the aqueous film for the pH-regulation. The root cells thus to some extent create their own pH-environment. The respiration generally tends to increase the H-ion concentration in the aqueous film. If on the other hand, the com-

position of the solution favours the absorption of cations, e.g. in solutions of K_2SO_4 , the LcH-value will increase, despite the respiration. In this case the phase boundary potential of the root decreases, in the reverse case (= decrease of LcH) the potential difference increases.

An example of a decrease in $\log \frac{ZcH}{LcH}$, brought about as a predominant effect of respiration, is given in fig. 15. The ends of roots of three plants were dipped into c. 3 cc of 0.0001 n KCl. In 30 minutes the negative potential decreased to 20—30 mv. After renewal of the solution, the potential rose again to c. -50 mv. This regulation of the LcH-value amounts to c. 0.6 pH-units. In KCl the cations are absorbed somewhat faster than the anions and the acidifying action of the remaining anion is increased by the emitted HCO_3 -ions.

If, now, the occurrence of an aqueous film of special composition is avoided as far as possible by means of a flowing solution, the decrease of the negative root potential nevertheless endures in potassium solutions, but not in salts of Li, Na and Ca. The registrations of the galvanometer deflections imply a continuous current flow through the plants. This current is very weak, because the resistance of the KCl-tubes, which serve as junctions, is very high, some 10^7 ohms. The current is then c. $4-8 \times 10^{-9}$ amperes. Control experiments show no influence of this weak current on the boundary potential. Some experiments, in which the potential was

Table 5.

Time series in different chlorides. Three plants in each experiment.

Time	0.001 n KCl	0.001 n LiCl	0.001 n NaCl
4 minutes	-84 mv	-97 mv	-80 mv
40 "	-50 "	-97 "	-91 "
75 "	-24 "	-93 "	-84 "
120 "	- 9 "	-87 "	-83 "

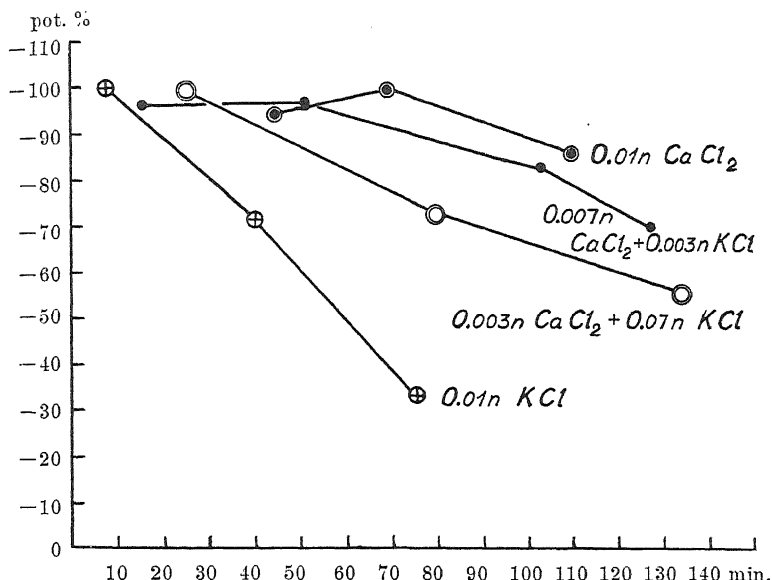


Fig. 16. The time course of root potential in solutions of KCl, CaCl₂ and combinations of these salts.

measured discontinuously at long intervals by means of a Wheatstone bridge device, give quite similar results (see table 5).

Special series were devoted to combinations of Ca- and K-salts. One of these is visualized in fig. 16. The potentials are here taken as percentages of the maximum value. The curves demonstrate clearly that the potential declines rapidly in 0.01 n KCl. After 75 minutes it amounts to only 33 % of the initial value. A mixture of one part of CaCl₂/2 and two parts of KCl (still 0.01 n) raises the end value to 78 %, or 55 % in 130 minutes. If equal parts of CaCl₂/2 and KCl are mixed, the decline is still more limited. In pure CaCl₂ no decline at all can be noticed in the boundary potential.

Experiments with complete nutrient solutions show that a certain negative charge of the root as compared with the solution is the normal state. In freshly prepared nutrient solution wheat roots attain a potential of -40 to -65 mv,

which corresponds to a ZcH -value of c. 10^{-6} ($ZpH = 6$) (see LUNDEGÅRDH 1938 a and 1939 a). It was found that measured potentials refer chiefly to the boundary between root and solution, because changes in the composition of the solution cause immediate changes in the potential. If the time of observation is extended, absorbed ions are to a certain extent transported to the inner cells, and the possibility cannot be excluded that observed changes in the potential apply to inner boundaries too. This question must be left open. The comparatively rapid fall in the potential curve in potassium solutions indicates, in my view, that primarily the boundary between the solution and the root is affected. As a negative potential of c. -50 mv is the normal state of the root surface, the decline in pure potassium salts indicates a »*degeneration*» of the protoplasmic membranes. I recall here the previous discussion on the detrimental effect of an intensified hydration of the membrane, which seems to result in a decrease of the H -ion activity of the membrane. Now the K -ion is known to intensify the hydration of the protoplasm, to make it more »liquid», and the decrease in potential, which indicates a decrease in the ZcH -value, seems to be a consequence of this circumstance.

Pursuing our line of thought, it is to be assumed that the curative effect of calcium on the properties of the protoplasmic membrane is caused by the lowering of the hydration. Manganese, too, raises the surface potential of the root (see LUNDEGÅRDH 1939 b). Roots grown in nutrient solutions free from Mn gave c. 30 mv lower potential than normal. The normal state of the protoplasm seems to require an assortment of a number of nutrient cations adsorbed at the surface. Of these, apparently, Ca is the dominant one, but also Mn must be present in small amounts, at least in wheat roots. The necessity for a small amount of Mn indicates that the cations in the protoplasm do not act exclusively physically, i. e. by means of their effect on the hydration, the charge etc. of it, but also chemically, as links in metabolic processes.

The work by BURSTRÖM (1939 a) on the manganese effect on nitrate reduction shows that adsorbed Mn-ions also promote the reduction process. LUNDEGÅRDH (1939 b) found Mn to be a catalyst in the fundamental respiration of the cell. This catalytically active manganese seems to be only adsorbed, i. e. bound in the same manner as cations in a colloid. BURSTRÖM found that the chemically active Mn was inactivated by other cations under circumstances identical with those observed in the exchange of adsorbed ions. It was found (LUNDEGÅRDH 1939 b) that the effect of Mn starts shortly after its addition. The previously mentioned fact that the adsorbed Mn-ions influence the surface potential too, seems to indicate that the catalytic action is developed from Mn-ions in the surface of the cell. This assumption throws an interesting light upon the complicated structure and function of the membrane. Just the twofold action of Mn-ions, as regulators of the surface potential and oxidation-reduction systems, indicates that the membrane is built up of molecular complexes or large molecules, which take an active part in chemical processes. We seem here to meet with a real example of chemical processes in surfaces, which have played a certain rôle in the discussions of several workers in physical chemistry.

Not only cations influence the electrical charge of the protoplasmic membrane at longer exposure. Anions, too, participate in the surface effects. Fig. 12 shows some curves from registerings of the root boundary potential in diluted pure acids. The HNO_3 -curve deviates decidedly from the coincident curves for HCl and H_2SO_4 . Nitric acid has a strong eliminating effect on the potential. Earlier experiments (LUNDEGÅRDH 1937) show that the root respiration increases greatly in pure acids. In the following pages we shall see that respiration lowers the potential. This fact might be one cause of the depressed course of the potential curves of pure acids, but it seems not to be sufficient to explain the specific behaviour of roots in nitric acid.

Among the acids tested was also boric acid. Detached

roots, which had been treated 20 hours with 10^{-4} m H_3BO_3 , showed in 10^{-4} n HCl a potential of -49 mv, while controls, which had been treated with aqua dest., showed -67 mv (average of 8 measurements). Pure acids of $\text{cH} = 10^{-4}$ n usually have a slightly lowering effect on the potential of the root tip, if the roots are treated with them for 20 hours (see also fig. 12). Owing to the low dissociation the cH -value of a 10^{-4} m H_3BO_3 -solution is comparatively low, but the possibility cannot, of course, be excluded that part of the boron effect is a cH -effect. For that reason the experiments were repeated with Na_3BO_3 . A 0.001 n solution of sodium borate had a pH of 6.88; a 0.001 n KCl solution had $\text{pH} = 6.78$. When intact seedlings had remained for 42 hours in these solutions, the potential of the roots was determined in 0.001 n LiCl. The boron-roots showed a potential of -67 mv, whereas the potassium-roots attained -108 mv. In 0.0001 n HCl the values were -59 mv and -85 mv respectively. The conclusion is to be drawn that boric acid causes a decrease in the ZcH -value of the roots. These results are interesting, because boron belongs to the minor elements of presumptive catalytic action. I recall here the well-known fact that numerous investigations prove the necessity of minute quantities of boron for the normal growth and development of several plants, preferably leguminosae.

Conclusions. The surface of the protoplasm — as investigated in roots of cereals, especially wheat — shows many analogies to the monolayers of fatty acids, lecithin etc. The exchange of cations for H-ions, the antagonism of cations and the effect of the adsorbed cations on the physical properties of the layer are phenomena with many analogies.

If the protoplasmic surface shows, in many respects, the character of a monomolecular layer, this layer must however be built up of molecules of different kinds. The observed electrical charge of the outer phase boundary corresponds to a maximum ZcH of c. 10^{-3} n in the apical part of wheat

roots, and the constancy of this H-ion tension at LcH -values up to about the same figure prove the existence of molecular groups of high dissociation power, which are plotted as islands in a layer of non-dissociated molecules. The maximum ZcH -value is at the same time an expression of the maximum exchange capacity of the membrane for cations.

The number of positive valencies in the surface layer is presumably low in comparison to the ZcH_{\max} value.

All potential measurements, performed soon (in c. 2 minutes) after a change in the bulk phase refer to the surface layer of the organ. The slow change in the potential in certain solutions indicates, on the other hand, the existence of sublayers and chemical processes going on deeper in the organ — or possibly deeper in the surface cells. These processes can lead to a change in the surface layer, e.g. an increase or a decrease in the number of negative valencies in the E -boundary. The properties of the surface layer are therefore not fixed and unchangeable but are partly a product of metabolic processes, e.g. respiration intensity (see below) unceasingly proceeding in the sublayer and in the bulk of the protoplasm. In addition to that, the changes in the physical properties of the surface layer, brought about by adsorption of ions of specific hydration etc., induce alterations in the layer itself. The »degeneration» of the layer, caused by the predominance of potassium ions, is an example of that. As a general reservation as regards interpretations of these slow changes in the behaviour of the surface layer, we must lay stress upon the fact that it is very difficult to determine whether a slow alteration is caused e.g. by a turning over of molecules (cf. LANGMUIR 1939) within a monolayer, or by chemical processes induced in deeper layers of the cell or in underlying cells. The reservation also extends to a possible collaboration of phase boundaries in deeper layers of an organ.

9. The mechanism of ion absorption.

Investigations on the characteristics of the protoplasmic membrane described in the preceding chapter support the assumption that absorption of neutral salts is not a permeability problem in the old sense of this conception, but involves ion reactions (ion »adsorption») in the membrane. H-ion activity, maximum adsorption capacity and electrical potentials in the E-boundary of the cells here reveal different sides of the same phenomenon: the chemical activity of the membrane. The first stage of the salt absorption is, according to this theory, the binding of ions in the membrane.

Cations are adsorbed until an equilibrium is attained between potential and exchange. The thermo-dynamical expression for the equilibrium would then be that metallic cations are exchanged for H-ions, until the work in moving one metallic cation from the solution to the membrane is equal to the work in moving one H-ion from the membrane to the solution. The process is approximately represented by the following equations:

$$-E = \frac{RT}{nF} \ln \frac{ZcH}{LcH}$$

and

$$ZcH = k \cdot [LcM]^{-a} \quad (\text{see p. 264}).$$

Thus

$$-E = \frac{RT}{nF} \ln \frac{k \cdot [LcM]^{-a}}{LcH} \quad (7)$$

or

$$-E = \frac{RT}{nF} \ln \frac{k}{LcH \cdot [LcM]^a} \quad (8)$$

The second equation is an empirical approximation of the cation exchange in solutions of c. 10^{-1} down to c. 10^{-4} n (see p. 264). Equation (7) consequently gives the interrelations of cH and cM in the solution in respect of their influence

on the cation adsorption. It shows that that O-potential depends on both the acidity and the salt concentration of the bulk phase and is lowered, if either of these increases.

The O-potential is an expression of the deficit in saturation of the cell surface with metallic cations. In normal nutrient solutions, the potential usually attains c. 50—60 mv, which corresponds to a ZcH value of c. 10^{-6} ¹. Because in wheat roots $ZcH_{\max} = c. 10^{-3}$, the neutral salts thus cause a partial neutralization of the protoplasmic surface. One would expect that an elimination of the rest of the O-potential would be brought about by ion exchange on the inside of the monolayer. But it seems to be a normal state for it to carry a certain negative charge. This fact points in the direction of metabolic processes being the cause of the persistency of the O-potential. The physical properties of the surface layer are also important, as the membrane seems to require a sufficient rigidity to resist the mechanical forces which the potential involves. We have learned that too much potassium in the membrane lowers its resistance. We return below to the discussion of potential and metabolism.

The normally negative values of E being a reality, as far as roots of cereals are concerned, the conditions for the absorption of cations and anions from the bulk phase are fundamentally different. Whereas metallic cations in diluted solutions — a complete discharge of the membrane requires abnormally high concentrations (see fig. 5) — always move in direction of the potential gradient, *the anions are repelled from the negative surface and an extra supply of energy is needed to overcome the resistance.*

This conclusion holds for all roots which, like wheat, are normally negatively charged in nutrient solutions. Oats and barley show similar ZcH_{\max} -values as wheat. In rye a ZcH_{\max} -value of c. 10^{-4} was determined. For that reason this cereal will absorb anions more easily than the other

¹ Potentials of this magnitude were also found in large cells (cf. BLINKS 1927, JACQUES, 1936).

cereals. Theoretically one can imagine uncharged membranes or membranes with positive charges; it will in each individual case depend upon the activity of the acid components of their protoplasm. A normally positively charged membrane will of course attract anions and require extra energy for the absorption of cations. Further investigations will perhaps reveal the existence of such membranes. I call attention here to the works of KROGH (1938) and HUF (1936) on salt absorption in the frog skin. Our present investigation is limited to the normally negatively charged, »acid» membranes of the roots of cereals.

The charge of the membrane influences not only the absorption of inorganic ions but also that of organic ones, e. g. dyes. YAMAHARA and NOMURA (1939) have recently investigated the influence of neutral salts on the absorption of acid dyes. They are unable to give an exhaustive explanation of this phenomenon. From our point of view, it is however evident that the neutral salts depress the ZcH -value and consequently make the protoplasm more alkaline — or less acid respectively — so that the ions of the dyestuff are less hindered by a negative membrane charge than in the absence of metallic cations. YAMAHARA and NOMURA have also observed that the entrance of the acid dyes is supported by a rise in the LcH -value. Also in this case our theory provides the answer: The height of the negative charge of the membrane is determined by the relation $\log \frac{ZcH}{LcH}$. Every rise in LcH will therefore diminish the negative charge of the membrane, or respectively increase its positive charge, and thus remove or diminish the resistance to anion absorption. If the ions of a dyestuff are too large to enter the membrane through intermediate adsorption (ion exchange), the uptake will be absolutely checked in the dissociated state. This is the case with rhodamin, according to DRAWERT (1939). Both on the acid and alkaline side of the i. e. p. the absorption is zero. But the neutral molecules seem

to be dissolved in the membrane substance and in that way enter the cells.

For the right understanding of the absorption phenomena, it seems to be important to realize that, whereas the cations are exchanged for H-ions in the surface layer *the anions too must be absorbed in a similar way, i. e. through exchange for OH-ions or other exchangeable anions*. An independent wandering of electrically charged ions is impossible: at each interphase, at a given moment an equal amount of anions and cations must be present. An exchange mechanism for cations, therefore, postulates an exchange mechanism for anions too. We concluded from potential measurements with salts of different anions that positive valencies are present in the membrane, whereas, in much smaller amount than the negative valencies, ZcOH_{max} is presumably much lower than ZcH_{max} or: the exchange capacity owing to anions is much lower than that owing to cations.

If there is equilibrium between the surface layer and the bulk phase, there will be no tendency for ions to enter the membrane or be expelled from it. This equilibrium will — in normal nutrient solutions according to our previous conclusions — be characterized by a large amount of metallic cations (ZcM) in comparison with a small amount of hydrogen ions (ZcH), furthermore a comparatively small amount of anions of strong acids (ZcA) and hydroxyl ions (ZcOH). Owing to the always present carbon dioxide there will probably also be a very small amount of HCO_3 -ions adsorbed in the surface layer. This is the *first stage of absorption*.

The *second stage of absorption* is the further movement of the ions, which have been adsorbed by the surface layer, into the protoplasm and the pulp of the cell. All the cells of the absorption zone in the roots contain cell sap, surrounded by a protoplasmic layer. The salts are generally accumulated in the vacuoles. But in a compact organ, which is built up by thousands of such cells, there is a general movement of the salts absorbed in the epidermis cells, in a direction towards

the central vessels and further upwards through these. A group of cells in the region of young root hairs (see p. 286) will function as a »symplast» (p. 251), and the accumulation of salts in the sap of the single cells will then be transitory. The aggregate of the cells will function as a bulk of living substance comparable to the protoplasmic layer in a single cell, which separates the outer diluted solution of nutrient salts and the more concentrated cell sap. The accumulation work will be performed by the protoplasmic layer, and schematically one can speak of the O-level as the boundary between the nutrient solution and the protoplasm, and the I-level as the boundary between the protoplasm and the cell sap. In an absorption organ, like the root, the O-level is the large contact area between the solution and the epidermis, and the I-level is a more diffuse boundary between root parenchyma and central vessel system. Each single cell is probably integrated in a large accumulation and transport system but there seems to be no reason to doubt that the moving forces are the same as in the case of an isolated cell.

If no accumulation at all existed, the above-mentioned equilibrium of adsorption in the O-level would persist. Accumulation work is necessarily connected with a continuous disturbance in this equilibrium. If we apply the principle of membrane equilibrium to the protoplasmic surface because the acid components of the membrane behave as large, non-diffusible anions (cf. K. H. MEYER 1935) the relations of anions and cations in the E-boundary will be represented by the equation

$$\frac{ZcH}{LcH} = \frac{ZcM}{LcM} = \frac{LcA}{ZcA} = \frac{LcOH}{ZcOH} = C. \quad (9)$$

$$58 \cdot \log C = -E_{mv} \text{ at } 20^{\circ} \quad (10)$$

If LcH is put equal to 10^{-7} C is normally > 1 in roots, mostly = c. 10, corresponding to $-E = \text{c. } 50\text{--}60 \text{ mv.}$ In a neutral solution $LcM = LcA$ the value of ZcA will, therefore,

normally be only c. 1/100 of ZcM . Assuming a low number of positive valencies in the protoplasmic membrane these will nevertheless be saturated with A-ions even at low salt concentration and a pH of 7. We return below to the question of LcH in nutrient solutions, which, according to the »self-regulation» of the roots, tends to a higher value than 10^{-7} .

If LcM, LcA, LcH and LcOH are regarded as constant, which is approximately true in a flowing solution, a disturbance in the equilibrium, resulting in a further uptake of the salt MA, can be brought about by changing any one of the Z-components. If, e. g., M-ions move towards the I-level, new M-ions will be attracted to the membrane. *An onesided accumulation of cations will go on if acids are produced in the I-level.* Organic acids frequently invade the vacuoles, which normally have a higher cH than the bulk of the protoplasm. In the I-boundary there will be, for that reason, a tendency for the protoplasm to throw out metallic cations in exchange for H-ions, i. e. the reverse of what normally happens at the O-boundary. Metallic cations will be continuously removed from the surface layer and transported to the membrane of the vacuole. The mechanism of this interplasmatical movement is of course hypothetical, but microscopical and especially ultramicroscopical observations teach us that particles move rapidly in all directions, even if no mass streaming of the protoplasm occurs. Pure diffusion would of course also transport the ions from one level to the other, but it would take more time than that which is actually observed for the accumulation work. In the production of exchangeable H-ions in the I-level also the carbon dioxide, produced in respiration processes, takes part, so that bicarbonates are produced. OSTERHOUT describes a model for cation accumulation, in which carbon dioxide serves as acidifying agents at the »I-level».

The one-sided accumulation of metallic cations will cease as soon as the acid components in the protoplasm and the

acids produced in the cell, or organ, or plant, are neutralized. Some cations, such as NH_4 and Fe, disappear as ions, when they enter as components into organic compounds. Part of Ca and Mg is precipitated in inorganic or organic salts etc. But the majority of cations, especially potassium and much sodium, calcium and magnesium, are accompanied by inorganic anions, accumulated from the O-level.

Also a number of anions enter the organism in the direction of the diffusion gradient. Nitrate ions disappear in the formation of proteins, and similarly with sulphate ions. In respect of nitrate the reduction clearly creates a very favourable situation, because the acid is transformed into a base. Phosphate, on the other hand, is combined with organic radicals, but usually keeps its anionic character. Nevertheless inorganic phosphate is frequently accumulated, and the same is the case with chloride. Nitrate and sulphate are also frequently accumulated and transported as intact anions. It is, therefore, obvious that the living substance possesses a special mechanism, by means of which *inorganic salts are moved against the concentration gradient*. This mechanism very probably acts also in those cases when ions are quickly transferred into non-dissociated compounds or ions of reversed sign. I mean that the accumulation mechanism will probably work independently of the direction or steepness of the diffusion gradient, simply as a mechanism for rapid transport of ions from one level to another. Observations on the accumulation and reduction of nitrate speak directly in favour of this assumption.

Owing to the ion equilibrium in the phase boundary, the ion products in the surface layer will be equal to the ion products in the bulk phase. We postulate here a free mobility of the ions in the exchange process and ideal conditions in general, thus unlimited space for exchange etc. In reality the exchange capacity is limited, and we know little about the activity. The applicability of the Donnan principle is also connected to the assumption of free mobility of salt ions

within the membrane which is somewhat doubtful in the light of our experimental results (cf. p. 246).

$$\text{If } ZcA = \frac{LcA \cdot LcH}{ZcH}$$

we find that the concentration of anions in the O-level increases when LcH increases. Consequently *all circumstances which tend to increase the H-ion activity in the nutrient solution would promote the anion absorption*. But this is not always the case (cf. p. 241). Another question is the cation absorption: It is a well known fact that a cation of high mobility, e.g., potassium, promotes the uptake of nitrate etc. (LUNDEGÅRDH 1932, BURSTRÖM 1934). A rapid cation absorption from the nutrient solution tends, of course, to increase LcH. Here we touch upon a fresh side of the accumulation problem: the rate of absorption.

In regard to the cations, which, to much larger amounts than the anions, invade the surface layer and saturate the negative valencies in it, the rate of accumulation is fairly independent of the LcM-value, if convexions in the solution provide enough material. Potential measurements in salt solutions show that all kinds of cations are adsorbed, and that the power of adsorption increases with the charge and diameter of the ions. On the other hand, determinations of the gross absorption of cations in the plant show that ions of high adsorption power, e.g. Ca, are accumulated to a much smaller extent than ions of low adsorption power, e.g. potassium. The ion mobility in an electrical field thus seems to determine the transport and accumulation within the plant, *and the transitory adsorption in the surface layer (= ZcM) is by no means a decisive factor for accumulation capacity*. We have seen that, even in very diluted solutions the cations invade the majority of the negative valencies of the membrane. The first stage of absorption accordingly represents an »overdimensioned» availability of cations. This conclusion

holds good in respect of single salt solutions or well-balanced nutrient solutions. In other cases the ion antagonism is probably capable of limiting the supply of elements by depressing this first stage of absorption. As regards the anion absorption, the total amount of adsorbed cations is the deciding factor. And the value ΣZcM , which includes the whole assortment of adsorbed cations, will always be very large in comparison to ZcH , if the plants grow in diluted solutions of salts of any kind.

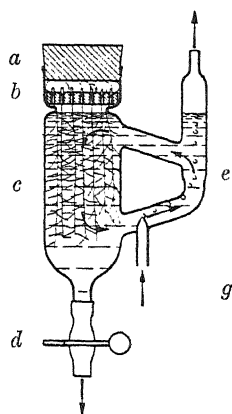
A factor which tends to augment LcH is the carbon dioxide. All cells respire, and the expelled CO_2 neutralizes as HCO_3^- ions a possible excess of metallic cations with rapid anion absorption. Also the acid H_2CO_3 owing to its dissociation, directly increases LcH (LUNDEGÅRDH and BURSTRÖM 1933 a). Some investigators suggest the possibility of an exchange of the anions in the solution against HCO_3^- ions from the cell (OSTERHOUT 1936). An exchange postulates a detachment of ions from the surface layer, but it seems doubtful whether the positive valencies in this layer are saturated by HCO_3^- ions instead of OH^- ions, since the decidedly acid nature of the membrane restrains the dissociation of H_2CO_3 . According to SHEDLOVSKY and MAC INNES (1935) the first ionization constant of H_2CO_3 is 4.31×10^{-7} at 25°C ($\text{pK}_1 = 6.37$). *The influence of the expired CO_2 is probably restricted to its entrance as an acid into the chemical balance.* Earlier experiments (LUNDEGÅRDH and BURSTRÖM 1933 b) show that the anion absorption is promoted by an artificial increase of the CO_2 -content in the solution.

10. The »anion respiration». Interaction of anions and cations. The coefficient k .

In studying the relations between the respiration of roots and the absorption of anions and cations, LUNDEGÅRDH and BURSTRÖM (1933, 1935) established that the amount of absorbed anions showed a marked relation to the quantity of carbon

dioxide given off by the respiration. VAN ELJK (1939) recently confirmed what he calls the »LUNDEGÅRDH principle», i. e. a quantitative relation between salt absorption and respiration, and he criticizes the view advanced by STEWARD and HOAGLAND that the respiration is only a sort of unspecific condition for the salt uptake.

Fig. 17. Circulation vessels, as used for experiments in anion respiration. 15 seedlings are carried by a cork holder *b* and the roots are surrounded by the salt solution in the container *c* (c. 100 cc). In most experiments the stalks are cut off above the seeds and the vessel is closed by a rubber stopper *a*. If air (or oxygen) is let in through *g* the bubbles will escape through *e* and cause a circulation of the solution as indicated by the arrows.



Experiments on the relation between salt absorption and respiration were carried out along two lines. In one series of experiments groups of seedlings were observed in parallel: the groups were placed in solutions of different salts of different concentrations (fig. 17). All other conditions, temperature, air supply, illumination etc. were constant. The plants were grown under controlled conditions, and the usual variations were eliminated by using groups of 15 plants for each experiment (see LUNDEGÅRDH 1932, 1933, 1935, 1937). In other series of experiments the salt conditions were changed during the experiment and the effect was observed. In this case the influence of variability from the individuals is excluded.

Experiments as shown in table 6 were repeated several times (see also LUNDEGÅRDH 1937), always with the same results: 1. if the roots are kept under otherwise constant conditions, the respiration is increased when the salt absorption increases, e.g., owing to a higher concentration of

Table 6.

Salt absorption and respiration. Groups of 15 plants in each experiment. mE = milli-equivalents.
mM = millimols.

Salt	absorbed		total respiration	time
	cations	anions		
0.0005 n KNO_3	0.060 mE	0.076 mE	0.305 mM	0—2 hours
0.0050 " "	0.066 "	0.159 "	0.409 "	2—4 "
0.005 n $\text{Ca}(\text{NO}_3)_2$	0.040 "	0.086 "	0.362 "	0—3 "
aqua dest.	—	—	0.249 "	3—6 "

the solution; 2. if the roots come from distilled water in a salt solution, which is not too diluted, the respiration increases when the absorption begins; if the roots come from a salt solution in distilled water, the respiration decreases.

This general statement of a kind of quantitative relation between salt uptake and respiration is based on a very large number of careful experiments in this laboratory carried out during the past eight years. It was mentioned above that STEWARD, HOAGLAND and collaborators have not been able to demonstrate any quantitative relation between salt absorption (accumulation) and respiration. They believe that aerobic »metabolism» is an unspecific condition for salt uptake, and their findings are limited to the statement that the uptake ceases if oxygen is excluded, and starts again if oxygen is supplied. Their failure to find a more quantitative relation is probably due to inappropriate material. It has already been emphasized how very important is, for such experiments, to choose an object which is exclusively an absorption organ. All cells and all organs show aerobic metabolism, and if this fundamental respiration is too large in comparison with the oxidation processes, which are expected to be coupled with the salt absorption, the inevitable errors in the determination of anions, cations and carbon dioxide will tend to

obscure the »salt respiration». VAN ELJK (1939) has criticized at length the experiments of STEWARD etc. (see also LUNDEGÅRDH 1940).

Personally I avoid criticizing the results of other investigators, if I have not been in a position to repeat their experiments. As a general rule I think one ought to trust the reliability of published facts, if it has not been directly proved that the observations are wrong. This does not seem to be STEWARD's attitude. He criticizes, frequently and vigorously, the investigations of LUNDEGÅRDH and BURSTRÖM, whereas he fails to repeat their experiments or thoroughly to study the papers in which the experiments are described. I do not greatly believe in the value of polemics. I have, for this reason, hitherto abstained from answering STEWARD's attacks on my work. An article by HOAGLAND and STEWARD in *Nature* (1939) was answered in *Nature* (1940). Light is thrown on the polemical method of STEWARD (and HOAGLAND) in the following pages.

STEWARD (1935) claims in a review that »LUNDEGÅRDH and BURSTRÖM's view that there is a special respiration component due to salts (anions) is not supported by any data of which the writer is aware». If STEWARD (and HOAGLAND and collaborators) has not succeeded in the isolating of a component in the total respiration, which is coupled with the salt absorption, this must not necessarily be due to mistakes made by LUNDEGÅRDH and BURSTRÖM. It may also be due to the inappropriateness of the experiments carried out by STEWARD and HOAGLAND (see also the criticism of VAN ELJK, 1939).

It is surprising when an investigator claims that his own results give »excellent evidence» (STEWARD 1935, p. 539) of the view that LUNDEGÅRDH and BURSTRÖM's results are wrong, in spite of the fact that he has not tried to study their experiments. There are several examples of this strange over-estimation of his own results and opinions in STEWARD's publications. I confine myself to one more case. STEWARD (see CHANDLER 1939) has modified the method of RAMAGE for the spectrographical determination of alkali metals in extracts of plants. He describes his procedure briefly on two pages. Dates from which the accuracy of the determinations can be demonstrated, are not given. STEWARD claims, nevertheless, that »this method has obvious advantages over that used by LUNDEGÅRDH» (l.c. p. 65). The LUNDEGÅRDH-method for quantitative spectral analysis has been described in detail in

a large number of publications (from 1929 to 1939) and is now employed in several countries.

In striking contrast to his tendency to deny the value of the results of other workers stands STEWARD's incomplete knowledge of the contents of the papers by LUNDEGÅRDH and BURSTRÖM. He claims, for example, (STEWARD 1935 p. 538) that LUNDEGÅRDH and BURSTRÖM (1933) presume »that salt absorption regulates respiration because entering anions require excess production of bicarbonate». LUNDEGÅRDH and BURSTRÖM have never assumed anything of the sort. They have shown that a coupling exists between a respiration process in the roots and the absorption of anions. We do not know anything about bicarbonates in the roots. Bicarbonate in the solution which surrounds the roots is a phenomenon of secondary importance, which depends on the expired CO_2 and the cation:anion balance in the solution. A direct exchange of bicarbonate ions, produced within the root, against anions in the solution is very improbable and in any case quite hypothetical. This question has been thoroughly discussed in the papers by LUNDEGÅRDH and BURSTRÖM.

HOAGLAND and BROYER (1936 p. 490) admitted that »the objectives and technique of the two investigators (= LUNDEGÅRDH and STEWARD) are so dissimilar that a comparison of results is of limited value». HOAGLAND has unfortunately abandoned this moderate position and subsequently associated himself with STEWARD in his more unrestrained criticism (HOAGLAND and STEWARD 1939). Setting aside the polemical element in STEWARD's and HOAGLAND's publications, their results show that they essentially agree with LUNDEGÅRDH and BURSTRÖM in respect of the fact that the salt absorption (accumulation) in plants is coupled with aerobical exothermical processes in the cells (= respiration). One of the fundamental differences between the two schools is that HOAGLAND and STEWARD look on the »salt respiration» as an unspecific metabolic activity, whereas LUNDEGÅRDH and BURSTRÖM, on the basis of comprehensive series of combined experiments, have tried to go farther in the analysis of the processes involved. These authors showed in 1935 already that the respiratory processes, which are coupled with the salt absorption, can by means of different manipulations (varied oxygen supply, treatment with cyanide etc.), be separated from a more unspecific »fundamental respiration».

A second fundamental difference between STEWARD and HOAGLAND and LUNDEGÅRDH and BURSTRÖM lies in the postulation of the former investigators that cations and anions are both similarly

related to the respiration. HOAGLAND and BROYER (1936 p. 490) »are emphasizing that respiration reflects energy exchanges essential to accumulation by root cells of *both* cations and anions». LUNDEGÅRDH and BURSTRÖM have found, on the contrary, that cations and anions behave differently in respect of absorption and respiration, so that the conception of individual mechanisms for their uptake is well founded. I have not found any data in the publications by STEWARD and HOAGLAND which vitiate this view.

A third difference between STEWARD—HOAGLAND and LUNDEGÅRDH—BURSTRÖM is that the former authors deny any quantitative relation between salt absorption and respiration. VAN EIJK (1939) refers to the »LUNDEGÅRDH principle» when speaking of this difference. The LUNDEGÅRDH principle involves that some quantitative relation exists between the two phenomena, but this relation is not of a stoichiometrical nature. A further characteristic of the relation may be gathered from LUNDEGÅRDH (1937) and the present paper, which throws fresh light on the question.

In conclusion I would adduce the following with regard to the polemical methods of STEWARD and HOAGLAND: The fact that one investigator is unable to accept the results of another investigator must not necessarily imply that the latter's experiments are unsatisfactory or that his conclusions are wrong. I consider that remark is of general significance.

The experiments with large series of uniformly cultivated groups of plants, which were, under otherwise constant conditions, placed in solutions of different concentrations, were also repeated. Table 7 visualizes a series of experiments with potassium chloride. In experiment 1 the anion uptake was checked by a previous treatment with KCN (see below). In the remaining experiments the variation in ion uptake depends on the general variation in the nutrition conditions of the groups. The most important conclusion from the experiments is, nevertheless, the fact that the respiration, measured as CO_2 given off from the root systems, increases when the ion uptake increases. If the values for the anion uptake are plotted as a curve, we get a nearly straight

Table 7.

Ion absorption and respiration. 2 groups of plants (totalling 30 individuals) were placed in each solution. »Circulation vessels» were used. See LUNDEGÅRDH (1933, 1935, 1937) and fig. 17. Time 6 hours. Age of seedlings 3 weeks. 0.002 m KCl solution (c. 100 cc in each vessel, 200 cc per 30 seedlings) in No. 1—3, 0.004 m in No. 4.

No.	absorbed		given off Ca	total respiration
	K	Cl		
1	0.070 mE	0.034 mE	0.006 mM	0.6773 mM
2	0.067 »	0.059 »	0.0191 »	0.7981 »
3	0.084 »	0.088 »	0.0202 »	0.8306 »
4	0.160 »	0.115 »	0.0120 »	0.9600 »

line, the prolongation of which cuts the abscissa at some distance from origo (see fig. 18). This is the meaning of the empirical formula $R_t = R_g + k.A$. As VAN EIJK (1939) remarks, this formula ought to be supplemented by the introduction of a time factor, but for comparison of experiments during a fixed time this factor can be omitted.

In the research work at this laboratory, we have always laid stress upon an accurate determination of both anions and cations. Our large collection of figures, visualizing the uptake of each category of ions, renders possible the plotting of the respiration values against them separately. This procedure is the more necessary as anions and cations are seldom absorbed in equal amounts (see p. 239). In all our experimental series from the very beginning, the reader can readily observe that, whereas on an average the relation $\frac{\text{absorbed anions}}{\text{absorbed cations}}$ is fairly characteristic for each salt, the individual variation is undeniable. This depends partly on the previous cation content of the seedlings (see p. 256), and the variation of the cation uptake is frequently accompanied by a variation in

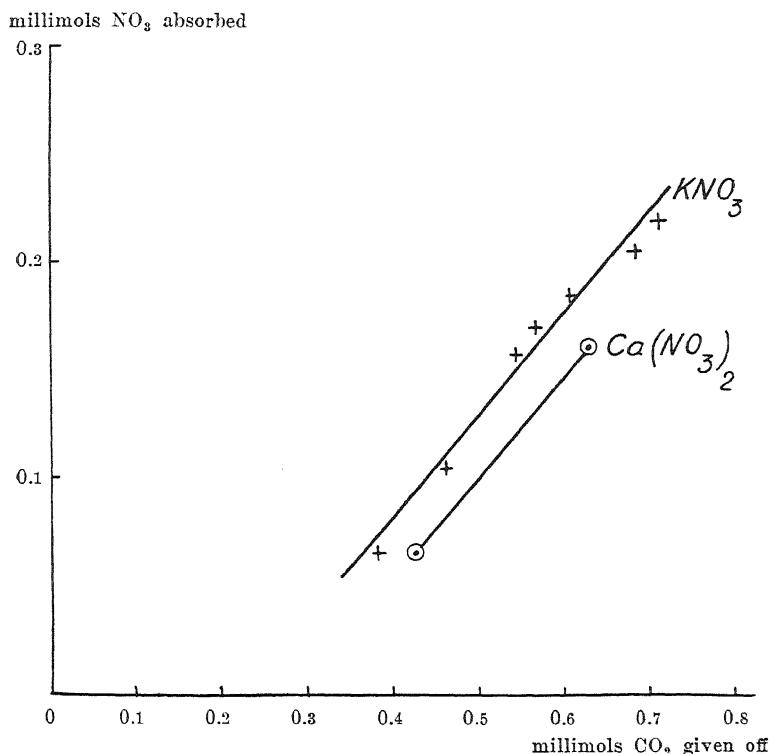


Fig. 18. Experiments in circulation vessels, showing the relation between absorbed amounts of NO₃ and the intensity of respiration of the roots.

exchanged other cations, e. g. calcium for potassium etc. It is feasible to charge the roots with cations (e. g. Na or Ca) and hence suppress a subsequent absorption of these ions from a solution of a single salt.

In our table 7 No. 1 and 2 both show about the same K-absorption. But the Cl-absorption for No. 2 is about twice that for No. 1. And the respiration here follows the anion uptake. In our earlier experiments we varied the cation of the salt and conducted series with NO₃ and Cl as anions.

In order to demonstrate how unjustified were the strictures of STEWARD and HOAGLAND from the very beginning, I repro-

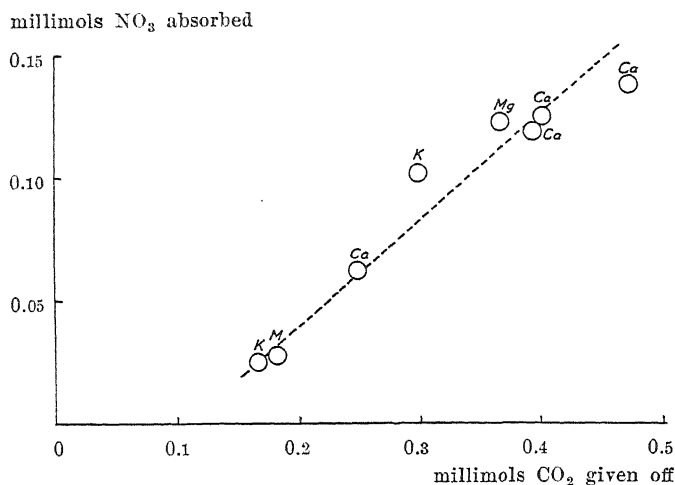


Fig. 19. From LUNDEGÅRDH and BURSTRÖM, 1933 b, table 1. The relation between respiration and NO_3 absorption in wheat roots. Nitrate series.

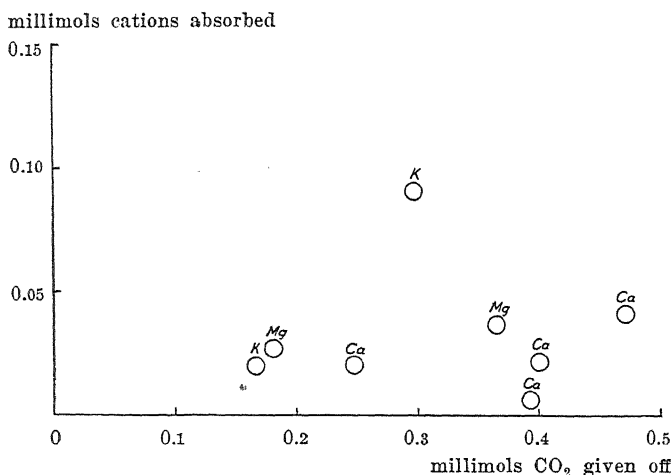


Fig. 20. As in fig. 19. Nitrate series. Respiration and absorption of cations.

duce in figs. 19—22 the results of the first of LUNDEGÅRDH and BURSTRÖM's series, published in 1933 (b). The figures of ion absorption and respiration are taken from table 1 in

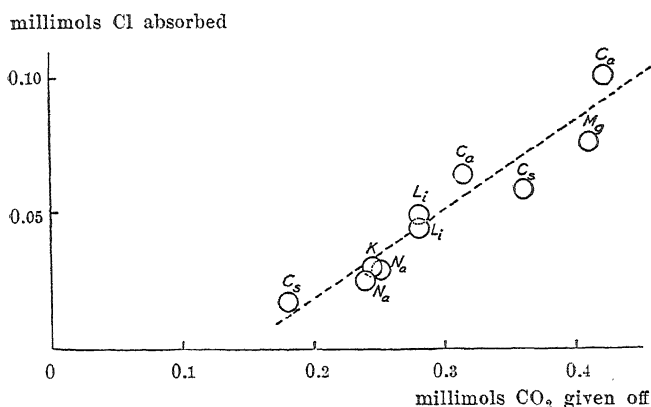


Fig. 21. From LUNDEGÅRDH and BURSTRÖM, 1933 b, table 1. The relation between respiration and Cl absorption in wheat roots. Chloride series.

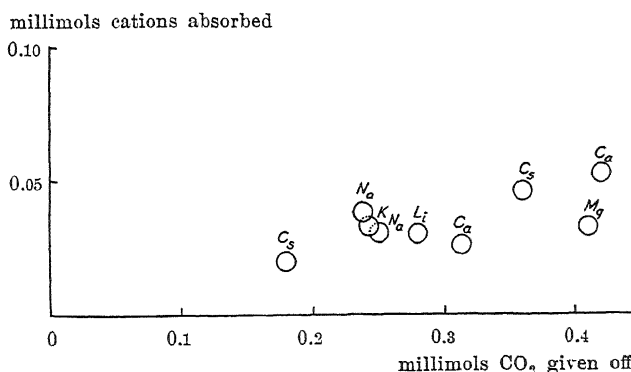


Fig. 22. As in fig. 21. Chloride series. Respiration and absorption of cations.

the paper mentioned. Fig. 19 shows the correlation between the uptake of NO_3^- ions from nitrates of Na, K, Ca and Mg and the total respiration of the roots. The NO_3^- values show clearly a »quantitative» relation to the respiration values. The corresponding values for the cation uptake from the same experiments (see fig. 20) are scattered and no quantitative relation between cation uptake and respiration is to be discovered. These experiments gave the first impulse to the theory of

anion respiration, and they show clearly that anions and cations enter largely independently of each other. The same will be seen from the chloride series (figs. 21 and 22). In this case a faint parallelism can be traced between anion and cation absorption, but this is not surprising in view of our discussions on the total ion balance. In respect of the Cl-series too, the quantitative relation between respiration and anion absorption is evidently more prominent than a relation between respiration and cation absorption. The extensive further studies, published 1937 (LUNDEGÅRDH 1937), give further, still more striking examples of the absence of a quantitative relation between cation uptake and respiration.

Experiments on anion respiration can be varied in different ways. In some experiments the behaviour of the roots in nitrate and in bicarbonate of potassium was studied. One of these gives:

0.002 m	KHCO ₃	K	abs. = 0.204 mM	CO ₂ evolution 0.6047 mM
0.002 m	KNO ₃	»	» = 0.204 »	NO ₃ abs. = 0.356 mM
				CO ₂ evolution 0.8836 mM.

The K-absorption in this experiment was exactly the same in both cases, but the respiration was much greater, when, too, an anion, NO₃, was absorbed.

The predominance of the anions in determining the respiration intensity can be demonstrated in many ways. Table 8 affords an example. Here, in two parallel experiments with KCl and KNO₃ approximately the same amount of cations, K, was absorbed. But from the KCl-solution 0.146 mM anions were absorbed, as against 0.356 mM from the KNO₃-solution. The respiration intensity was correspondingly larger in the latter case.

An evident influence of the anions on the respiration is at last noticeable in table 9. The larger acetate absorption in No. 2 is reflected in a higher respiration value, whereas the cation absorption was somewhat smaller than No. 1.

Recent and earlier experiments thus support the conclusion that a coupling exists between the uptake of

Table 8.

Absorption for potassium salts. One group of 15 plants in each case. Circulation vessels. Time of experiment, 6 hours.

Salt	absorbed			given off		total respiration
	K	Cl	NO ₃	Ca	Mn	
0.002 n KCl	0.206 mM	0.146 mM	— —	0.021 mM	0.004 mM	0.744 mM CO ₂
» KNO ₃	0.210 »	— —	0.856 mM	0.012 »	— —	0.884 » »

Table 9.

Absorption and respiration in potassium acetate (0.002 m).

Absorbed		given off Ca	total respiration
K	acetate		
1. 0.064 mM	0.144 mM	0.014 mM	0.835 mM CO ₂
2. 0.044 »	0.217 »	0.014 »	0.937 » »

anions and the respiration. From the point of view of energy the augmentation of the total respiration, which is due to the anion respiration, represents a quantum of energy, which can support a very high accumulation. The work of accumulation can be calculated from the equation

$$A = RT \cdot \ln \frac{c_1}{c_0} \quad (11)$$

Here c_0 is the concentration in the solution and c_1 the concentration in the cells, and in the organ respectively (bleeding sap etc.). Calculating with a concentration gradient $\frac{c_1}{c_0}$ of 100, which seems to be fairly normal, we get $A = 1.985 \cdot 293 \cdot 4.6 =$

= 2 680 g-cal., if $T = 20^{\circ}\text{C}$. In the anion respiration of 2—3-weeks old wheat seedlings, at least 1 mol CO_2 is produced for each absorbed and accumulated mol anion. The coefficient $k = \frac{\text{respiration in mols } \text{CO}_2}{\text{mols absorbed anions}}$ is usually even higher, for NO_3 1.5 to c. 2 and for Cl, Br, J, SO_4 more than that. The respiration material in the roots is glucose. 1 mol CO_2 represents the combustion of $1/6$ mol glucose, thus $\frac{674\,000}{6} = 112\,333$ g-cal. This quantity is more than 40 times larger than the energy needed for the 100-fold accumulation of a salt. We know, on the other hand, that the plant leaves frequently contain salts or cations respectively, in concentrations which are up to 1 000 times higher than in the substrate (LUNDEGÅRDH 1934 a, 1938 a). It is to be supposed that the transport from cell to cell involves very high concentration gradients, for the movement of salts in the organism can be very rapid. For that reason an energy relation between salt uptake and glucose combustion is not surprising. From the point of view of energy, it is of course quite unimportant whether the energy-expending process is coupled with the accumulation of anions or cations, or both. If the coupling exists between respiration and anion accumulation, the cations will be drawn in passively after the anions, as outlined above.

For seedlings of wheat 2—3-weeks old, at a temperature of c. $18\text{--}20^{\circ}\text{C}$., the value of the coefficient k was fairly constant, if, e. g., potassium nitrate or potassium chloride solutions were used. Our series with potassium nitrate gave mostly $k = 2$, and our series with potassium chloride $k = \text{c. } 3$. But these k -values are by no means to be looked upon as stoichiometrical. I have shown in previous papers (1937) that k varies with the cations. Generally k increases with the decreasing mobility of cations, and the increasing k -values for different anions ($\text{NO}_3 < \text{Cl} < \text{SO}_4$) point in the same direction: The amount of respiration necessary for salt absorption is an expression of the amount of

work required for the absorption and accumulation of a molar quantity of anions in a unit of time.

A few words will be needed to explain why also the anion respiration must be dependent on the cation absorption, in spite of the fact that the cations are spontaneously absorbed in the negatively charged roots.

The normally negative electric charge of the O-boundary is an obstacle to the anion absorption, and the work to be done in order to overcome this obstacle must be proportional to the height of the charge. We have seen that sulphate ions produce a lower negative charge in the O-boundary than Cl-ions, and these a lower charge than NO_3 -ions. It is, therefore, surprising that more respiration energy is needed to absorb one mol of sulphate than one mol of chloride or nitrate, and that for coefficient k we get the series $\text{NO}_3 < \text{Cl} < \text{SO}_4$. But the coefficient k is of course not only the indicator of the resistance for anion absorption in the O-boundary, but also for the further transport and accumulation within the root; this indicates a summation of the obstacle to the moving of anions, which depends upon magnitude, charge and hydration of the ions and acts along the same series, as above.

Now anions will not move one-sidedly, but must, at each step of their absorption and translocation, be accompanied by cations in equal amount. H-ions are always present, but an accumulation of H-ions acts, electro-chemically, strongly attractingly upon metallic cations, because of the fact that a rise of the H-ion concentration on the inner side of a phase boundary implies a negative electrical charge of it. By exchange of the H-ions with metallic cations on the other side of the phase boundary, the electrical charge will be eliminated, and this explains why metallic cations in a living tissue will always move against the highest H-ion concentration. An accumulation of inorganic anions by means of energy-expend-

ing processes will therefore always be accompanied by an accumulation of metallic cations.

If, now, the forwarding of metallic cations along the path of anion translocation encounters obstacles of any kind, the H-ion concentration at the I-boundary (= the place of accumulation) rises. The result is a rise in the negative charge in the I-boundary, which, on the other hand, increases the amount of energy needed for the anion accumulation (see above). This is apparently the reason why cations of high charge or large diameter indirectly raise the coefficient k . Such ions are certainly heavily adsorbed in the O-boundary (see p. 266), but their mobility in an electrical field is slow, and this fact is no doubt decisive for the mentioned effect. Because of their slow mobility in the protoplasm and the tissues, the exchange of such M-ions for H-ions in the I-boundary is retarded, and as a result the slow-moving cations will therefore inevitably be an obstacle to anion accumulation.

From what has been said here, it is obvious that the coefficient k does not give evidence of any stoichiometrical relations between the amount of absorbed anions and the amount of oxidized glucose. It is also evident that k is an expression of the total work performed in lifting anions from the O-level up to the I-level and accordingly is also dependent upon the manner of translocation (if the way is long or short) and upon the degree of accumulation etc. It cannot be expected that k should show any approximate degree of constancy unless the same plants, at the same stage of development and at the same temperature etc., are always used. Van Eijk found much lower k -values for *Aster tripolium* than our value for wheat. I have found that the k -values are lower for rye than for wheat. The k -values seem also to vary with the stage of development of the plant.

The zone of most intensive ion absorption is the lower 20 to 30 mm of the wheat roots. In the root systems of 2—3-

weeks old seedlings the long primary roots generally have a length of 10—15 cm. In order to demonstrate the ion absorption and respiration at different heights of the root system, this was cut into 30-mm long pieces, beginning from the tips of the longest roots. Table 10 shows the results.

Table 10.

Respiration and Cl-absorption in different parts of the root system of 2—3-weeks old wheat seedlings. The root systems of a group of 15 plants were cut into three pieces and the parts were enclosed in 25 cc flasks filled with a solution of 0.001 m KCl+0.0002 m CaCl_2 , saturated with oxygen. Duration of the experiment 40 minutes. After that time the consumed O_2 and absorbed Cl were determined.

$\text{O}_2 : \text{Cl}$		Respiration	Cl-absorption
7.1	Zone 0—30 mm (tip zone)	0.400 mM O_2 per hour and g dry weight	0.056 mM per hour and g dry weight
3.8	Zone 30—60 mm	0.134 " " "	0.035 " "
3.1	Zone 60—90 "	0.144 " " "	0.047 " "

The experiment, which was repeated several times, with the same main results, shows that the respiration intensity per g dry weight attains its maximum in the tip zone. This is not surprising, in view of the intensive metabolism in the growth zone. The relation O_2/Cl is rather high, which indicates an intensive fundamental respiration. The anion absorption decreases somewhat in the middle zone, 30—60 mm. Some roots were shorter than the rest and a small number of tips were therefore included in this zone too. The fundamental respiration is much lower than in the tip zone, so the bulk of the root parts no doubt belong to the out-grown tissues. The Cl-determination seems to show that anions are absorbed rather intensively also in the middle zone of the roots. In the oldest zone the Cl-absorption increases again, which is

probably due to the small roots of secondary order. The respiration is still much lower than in the tip zone.

Of course, absorption figures from cut root pieces always suffer from one main error: the pumping mechanism (see p. 251) of the roots allows part of the salts absorbed from the solution to escape again at the upper wound-surface, hence the true absorption values are lowered to an uncontrolled extent. BURSTRÖM (1939 b) investigated more closely the absorption for different heights of young wheat roots. He found a decided maximum in one zone close above the vegetation point, which includes the zone of young growing root hairs. This zone of maximum absorption seems to coincide with the zone of maximum negative electrical charge, as shown above, p. 284.

The relation between the adsorption power of the anions, the absorption velocity of anions and cations and the respiration intensity was studied in a large series of experiments with chlorides, bromides and iodides of potassium. Acetate of potassium was also included in this series. See table 11.

The experiments on which table 11 is based are of interest in several respects. From the point of view of experimental technique it is interesting to notice that the parallels correspond fairly well. Small variations are inevitable, for two sets of plants are never quite identical in behaviour, even if they are taken from the same culture vessel. Variations in the absorption power are certainly partly due to the fact that the upper 1—2 cm of the root systems are not submerged in the solution in the circulation vessels, and the size of this unsubmerged part may vary a little. Variations in the dry weight of the roots of identically cultivated plants might be caused by inevitable errors in cutting off the roots from the plants.

A comparison of the different experimental series shows that younger plants with smaller roots generally give a lower quotient $\frac{\text{CO}_2}{\text{anion abs.}}$. See No. 15—20 in comparison to 1—11.

Table 11.

Absorption experiments in circulation vessels (volume 100 cc) with slowly moving solution, No. 1—22, and in vessels with rapidly flowing solution (volume c. 2000 cc), No. 23—25. All figures refer to two groups of 15 plants each. Age of seedlings 2—3 weeks. Temperature 24° C. Salts in 0.002 m solution (No. 1—22), or 0.001 m (23—25). Duration of the experiments 6 hours.

No.	Salt	absorption		total respiration	$\frac{\text{CO}_2}{\text{anion}}$	dry weight g	anion cation
		potassium	anions				
1.	KCl	0.206 mM	0.146 mM Cl	0.744 mM CO ₂	5.1	1.52	0.71
2.	"	0.194 "	0.142 " "	0.717 " "	5.1	1.55	0.73
3.	KBr	0.138 "	0.117 " Br	0.772 " "	6.6	1.24	0.85
4.	"	0.122 "	0.110 " "	0.760 " "	6.9	1.23	0.90
5.	KJ	0.084 "	0.055 " J	0.808 " "	14.7	1.47	0.65
6.	"	0.098 "	0.062 " "	0.819 " "	13.2	1.52	0.63
7.	KCl	0.122 "	0.105 " Cl	0.802 " "	7.6	1.49	0.86
8.	KBr	0.084 "	0.128 " Br	0.920 " "	7.2	1.63	1.52
9.	"	0.092 "	0.144 " "	0.897 " "	6.2	1.56	1.57
10.	KJ	0.049 "	0.069 " J	0.847 " "	12.3	1.47	1.57
11.	"	0.058 "	0.080 " "	0.889 " "	11.1	1.34	1.38
12.	KCl	0.158 "	0.123 " Cl	0.843 " "	6.8	1.25	0.78
13.	KBr	0.163 "	0.110 " Br	0.847 " "	7.7	1.25	0.67
14.	KJ	0.074 "	0.074 " J	0.780 " "	10.5	1.20	1.00
15.	KCl	0.206 "	0.115 " Cl	0.581 " "	5.1	1.06	0.56
16.	"	0.190 "	0.121 " "	0.581 " "	5.0	1.01	0.64
17.	KBr	0.152 "	0.078 " Br	0.401 " "	5.1	0.99	0.51
18.	"	0.156 "	0.075 " "	0.397 " "	5.3	1.11	0.48
19.	KJ	0.116 "	0.054 " J	0.358 " "	6.6	0.94	0.47
20.	"	0.124 "	0.058 " "	0.383 " "	6.6	0.88	0.47
21.	K-acet.	0.120 "	0.254 " Ac	0.880 " "	3.5	—	2.1
22.	" "	0.104 "	0.243 " "	0.895 " "	3.7	—	2.3
23.	KCl	0.263 "	0.193 " Cl	0.945 " "	4.9	—	0.73
24.	KBr	0.280 "	0.179 " Br	0.918 " "	5.1	—	0.64
25.	KJ	0.189 "	0.079 " J	0.511 " "	6.5	—	0.42

Note. In experiments 12—14 the solution contained also 0.003 mols of glucose. In all the experiments small amounts of Ca (c. 0.02—0.03 mM) and Mn (c. 0.002—0.003) were given off. The nutrient solutions in which the seedlings were grown contained Mn.

But this higher »effectivity» is counterbalanced by lower values for the quotient $\frac{\text{anions}}{\text{cations}}$. This is chiefly noticeable in the KJ-experiments.

The circulation vessels (see fig. 17) were constructed for the purpose of securing an appropriate motion of the solution (LUNDEGÅRDH & BURSTRÖM 1933 b, LUNDEGÅRDH 1937). But experiments Nos. 23—25 show clearly that for maximum absorption a still more rapidly moving substrate is needed. Whereas in No. 23—25 only 0.001 m of salt was used, the absorption of anions is larger than the anion absorption in the circulation vessels, but not in relation to the respiration. Only iodide constitutes an exception. These experiments show that one must be very careful in generalizing from results of absorption experiments which are not made under comparable conditions.

The ions of the three halogenes have about the same mobility in an electrical field (Cl = 65.5, Br = 67.0, J = 66.5 l_A), but the adsorption in blood charcoal (see FREUNDLICH 1930) increases in the series Cl < Br < J. Judging from the behaviour of the series NO₃ < Cl < SO₄ (see p. 321) one would therefore expect increasing *k*-values. Table 11 gives only the relation $\frac{\text{total respiration}}{\text{anion absorption}}$ but it is obvious that Br must have about the same *k*-value as Cl, if the fundamental respiration is the same in both cases. Iodide, on the other hand, always shows a higher relation, the *k*-value here must, *mutatis mutandis*, be higher than for Cl. I fear that the calculation of a *k*-value will give a rather hypothetical result, because we do not know whether the fundamental respiration remains unaffected by the influence of the »unphysiological» anions Br and J. Some earlier observations give indications that the prolonged influence of ion uptake in solutions of single salts affect the whole metabolism (LUNDEGÅRDH 1937). Our observations on the special effect of unbalanced potassium solutions

on the protoplasmic membrane (see p. 289) have also demonstrated the complicated nature of the problem.

In table 11 we notice also the low quotient $\frac{\text{CO}_2}{\text{anion abs.}}$ in respect of acetate, which indicates a very low adsorptive power of the acetate ions. This is in accord with the effect of acetate upon colloids. In the present case the potassium acetate was to a large extent dissociated. The fact that the anion and the cation are absorbed in very varying quantities shows clearly that the acetate is absorbed as ion, not as salt molecule. Table 11 demonstrates again the principle of individuality of anion and cation absorption from a single salt.

Before we leave our table 11, one more thing ought to be discussed. I refer to the circumstance that the total respiration attains fairly similar values in all series with plants of the same age, whereas the ion absorption varies with the kind of ions. In experiments 1—14 the total respiration only varied between 0.717 and 0.920, whereas the anion absorption varied between 0.055 (for J) and 0.206 (for Cl). It looks as if the root system were characterized by a maximum respiration power and that, in solutions of sufficient concentration, this power was fully utilized. Owing to the larger work needed for the absorption of e. g. one J-ion in comparison to one Cl-ion, the maximum respiration power then only suffices for a smaller J-absorption. In rapidly flowing solutions the absorption seems to be promoted, and consequently the number of anions absorbed per time unit rises. The same result follows if the concentration of a slow-moving solution is increased.

If glucose is supplied, the total respiration increases. In table 11 glucose (0.003 m/lit.) was added to the salt solution in No. 12—14. As a consequence the total respiration rises (the dry weight in No. 12—14 was 1.20—1.25 g and the total respiration 0.780—0.847; in No. 3—4 [Br] we have the same dry weight but a respiration of 0.760—0.772), but the increase is limited to the fundamental respira-

tion and is not accompanied by increased anion absorption. This fact was confirmed also by other experiments. Sometimes the anion absorption decreased with the supply of glucose, e. g. in the following experiment:

0.005 m KNO_3 in 6 hours, 0.216 mM K abs., 0.611 mM NO_3 abs., resp. 1.036 mM CO_2 ; 0.005 m KNO_3 + 0.02 m glucose in 6 hours, 0.132 mM K abs., 0.426 mM NO_3 abs., resp. 1.239 mM CO_2 .

These observations on the effect of glucose, supplied from outside the roots, fully confirm the theory of anion respiration as a mechanism which is quite distinct from the fundamental respiration processes. Earlier experiments have shown that the root systems of our seedlings, which were grown under 18 hours' illumination of c. 20.000 Lux daily, contain an amount of glucose, sufficient to maintain a constant total respiration during at least 6 hours (LUNDEGÅRDH 1937). If a surplus of glucose, supplied from outside, stimulates the fundamental respiration, but not the anion respiration, this is probably due to the fact that the normal glucose content is not a limiting factor for the anion respiration mechanism. Only a restriction of the internal glucose supply will also affect the latter. Some experiments of this kind will be adduced below (p. 357). BURSTRÖM (1939) found that isolated young, rapidly growing roots from germinating seeds do not absorb anions in measurable quantities, unless glucose is supplied from outside. As a further proof of the correctness of the anion respiration theory, he stated that, in absence of glucose, cations were absorbed almost normally whereas the uptake of anions was checked. Consequently glucose — and respiration — are needed primarily for the anion uptake. The retarding effect of a large glucose supply to roots which have enough carbohydrates of their own is probably quite another problem, connected with surface reactions (see below, p. 256). The fact that the fundamental respiration obviously does not proceed with maximal speed, even in roots which are connected with vigorously

assimilating leaves, but is capable of consuming also an extra supply of glucose from outside, is of course important in respect of the experimental technique. One must be very careful in comparing results from individuals or groups of plants, which exhibit differences in age or nutrition. The anion respiration comprises a larger part of the total respiration in younger root systems than in older ones. A calculation of $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ always pre-supposes a very uniform plant material. In summary, our previous and present investigations render possible the following conclusions.

1. The fundamental respiration of the root systems is the sum of aerobic processes, which proceed independently of ion absorption. This group of processes is remarkably insensitive to cyanide and certain metallic poisons (LUNDEGÅRDH & BURSTRÖM 1935). The fundamental respiration increases beyond the «normal» rate when glucose is supplied from outside.

2. The anion respiration is rather sensitive to cyanide and is limited by the glucose supply, if this is lower than in vigorously growing 2—3-weeks old wheat seedlings. «Normally» such plants seem to contain enough glucose for the maximum rate of anion respiration, and no further increase is caused by an extra supply of glucose.

3. The anion respiration is coupled to the anion absorption: an increasing anion absorption is accompanied by an increasing anion respiration and vice versa. In aerobic life the respiration can be measured either as CO_2 or as O_2 , because in the aerobic roots $\frac{\text{CO}_2}{\text{O}_2} = 1$ (LUNDEGÅRDH & BURSTRÖM 1935). The coefficient $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ means on the other hand no stoichiometrical relation between the anions and the respiration mechanism.

4. The coefficient varies with the kind of anions

absorbed. It increases in the series (acetate) $< \text{NO}_3 < \frac{\text{Cl}}{\text{Br}} < \text{J} < \text{SO}_4$. In 2—3-weeks-old, vigorously assimilating wheat seedlings the k -values for NO_3 is c. 2 and for Cl, c. 3, if the root systems are surrounded by solution up to c. 1—2 cm from the seed. k is generally an expression of the total absorption and translocation work fulfilled in raising the anions from the O-boundary to the I-boundary. Therefore k must vary with the position of the I-boundary and owing to that seems to be lower for isolated root tips and old root systems with many secondary roots.

5. The coefficient k also varies with the kind of cations in the solution. It generally increases with the adsorption power of the cations, thus in the series $\text{Na} < \text{K} < \text{Ca} < \text{Sr} < \text{Ba}$.

6. Of a single salt the relation $\frac{\text{anions}}{\text{cations}}$ absorbed is usually approximately constant. If from CaCl_2 more Cl is absorbed in a more concentrated solution than in a diluted one, more cations are also usually absorbed in the former case. But if the absorption of cations is checked by feeding the plants in advance with calcium, the anion respiration still proceeds in relation to the amount of anions absorbed. Consequently it is not the amount of cations absorbed that is the cause of their influence on k , but their mere presence in the solution.

7. In distilled water the total respiration exceeds the calculated value of the fundamental respiration.

The only way to explain this strange semi-quantitative nature of the anion respiration and its modification by a number of accessory factors, seems to be to take an electrochemical view of the problem.

11. Electrochemical aspects of the absorption problem.

We have seen that the mere existence of cations in the solution regulates the surface potential of the absorbing organ.

Distilled water raises the potential to its maximum value. It was postulated that the entrance of anions requires an extra supply of energy to overcome the normally negative surface potential. We have assumed that this energy is supplied by the anion respiration process and that this process also works along the whole path of accumulation in the absorbing root system. On the electro-chemical theory of anion respiration it ought to be possible to demonstrate a causal connection between respiration and surface potential in the O-boundary of the root system.

A causal connection between potential of roots and respiration was found by LUND (1928) and by ROSENE and LUND (1935). These investigators have treated the problem otherwise than I have. As mentioned above, they speak of a rise of the positive charge in respiration. LUND and ROSENE assume the potential to be a consequence of the respiration. This assumption is well known from the investigations on the electro-physiology of nerves. LUND and ROSENE have measured the potential difference between single zones of *Allium* roots. As stated on p. 281, the charge in the O-boundary of roots varies with the distance from the root tip and if one electrode is applied to the zone of maximum negative charge, i. e. c. 10—15 mm from the tip in 10—12 cm long wheat roots, currents of reversed direction can be induced by applying the second electrode either to the base of the root or to the tip. Furthermore, if respiration is promoted more in one zone than in another and we place the root in nitrogen instead of oxygen the result in respect of relative charge will be different when the zone of maximum negative charge is most affected and when the base is so. We learn from this discussion that measurements of potential variations in single zones of a root have little value, unless the »topography» of the charge of the surface is known. In our experiments we treated the whole tip zone, including the zone of maximum charge, as a unit, because this zone shows a maximum of both ion absorption and respiration.

A large number of experiments was now started with the purpose of investigating the causal connection between aerobical respiration and the potential of the absorbing surface of the root.

The experiments were performed in the »potential vessels» (fig. 2). The plants, usually 3—5 seedlings, were held in position in the upper glass tube by means of dry cotton wool, so that the root-ends (25—30 mm) were submerged in the cylindrical container at the bottom of the vessel. The stalks were cut 1—2 cm above the seed and joined to a KCl-electrode by means of a cotton pillow soaked in 0.01 n KCl. By means of a tube and funnel the solution was poured into this container. At the bottom of the container was a tube for electrical connection to the KCl-electrode and a cock device for draining off the solution. Oxygen or nitrogen was introduced through a tube in the rubber stopper which closed the upper part of the vessel. Special experiments showed that the oxygen content in the solution (volume = 3 cc) was exhausted in a few minutes. By introducing nitrogen into the upper container, anaerobical conditions were soon attained. In some experiments the nitrogen was passed through the solution in order to accelerate the exhaustion of the oxygen. The nitrogen was carefully washed in alkalic pyrogallol solutions, in order to remove all traces of oxygen in the gas.

All experiments gave the same main result: In the absence of oxygen, the negative potential of the submerged root ends increased; in the presence of oxygen the negative potential decreased. I restrict myself to a graphical representation of two experiments (fig. 23—24). In the first experiment (fig. 23) the roots were at first treated with oxygen: after the change to nitrogen, the potential rises. In the second experiment (fig. 24) the treatment began with nitrogen for 120 minutes. In 10^{-4} n KCl a practically constant potential of c. -50 mv was obtained. After the change to oxygen, the potential fell to -36 mv in 60 minutes.

These results are interesting also in respect of the »dege-

nerating effect» of potassium in single salt solutions upon the surface potential (p. 290). This effect is retarded if oxygen is excluded.

In the experiments illustrated in fig. 23—24, the gases were let through both the solution and the upper container. Similar results were obtained if the gassing was restricted to the upper container. The same was the case with experiments in which the solution was isolated by a layer of liquid paraffin.

We have assumed that the O-boundary maintains a negative electric charge, apparently caused by the ionization (dealt with in the preceding account) and the subsequent surplus of H-ions after the partial adsorption of metallic cations. The effect of the respiration would then, from the electro-chemical view-point, be a further elimination of H-ions due to oxidative processes.

Experiments were also performed, in which potential and respiration were observed simultaneously. A few examples are reproduced in fig. 25. The potential of the root ends (measured in the potential vessels, fig. 2) and the respiration were determined at intervals of 30 minutes. The respiration was measured as O_2 -consumption. In other experiments the solution in the lower container (see fig. 2) was continuously emptied into a closed flask, and from time to time the O_2 -content was determined. The solution in the container was isolated by liquid paraffin and a special siphon device was used to effect the slow flowing of the solution through the container. See fig. 26.

In fig. 25 the potential attains c. -50 mv (a 0.005 m solution of $CaCl_2$ was used). The respiration in the first 30-minutes-interval is always low and rises after 60 minutes to a maximum value. This speaks in favour of a start-effect: the high potential seems to be the cause and the stimulated respiration the effect. But if the stimulated respiration then causes a partial elimination of the negative potential, the potential curve must decline when the respiration curve rises. This is

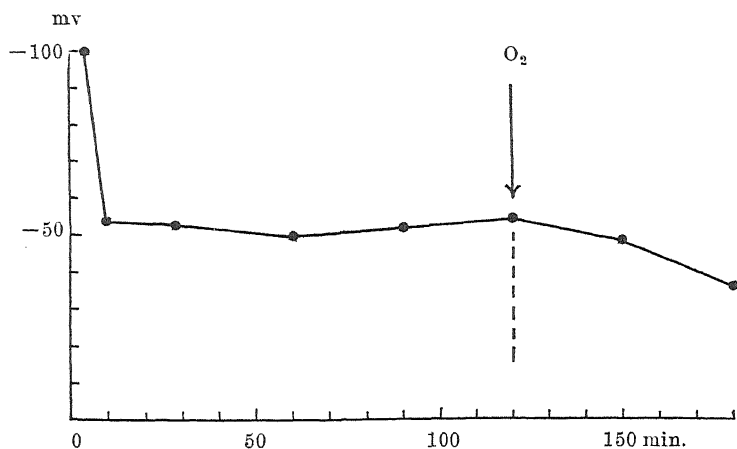


Fig. 23. Time course of the root potential in 10^{-4} n KCl. After an initial period of c. 10 min. («exchange potential») a fairly constant potential of -50 mv endures in nitrogen. After 120 min. oxygen was let in, and the negative potential now decreases.

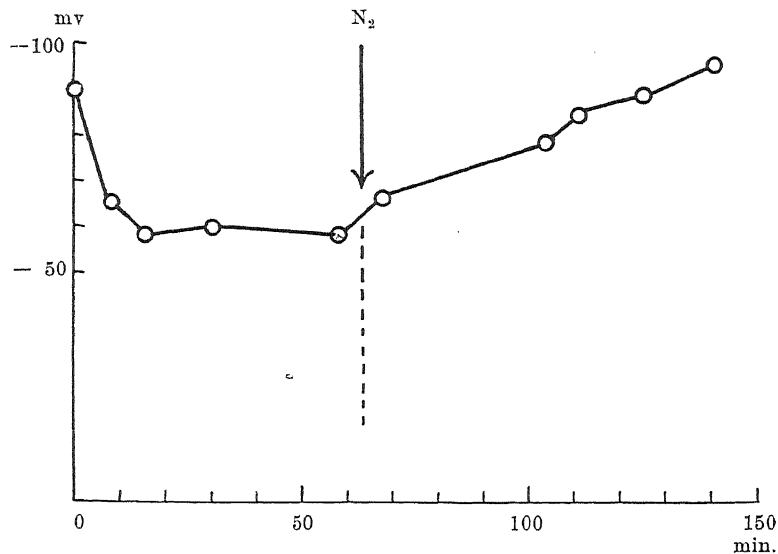


Fig. 24. This experiment started in oxygen. After the change to nitrogen the negative root potential increases.

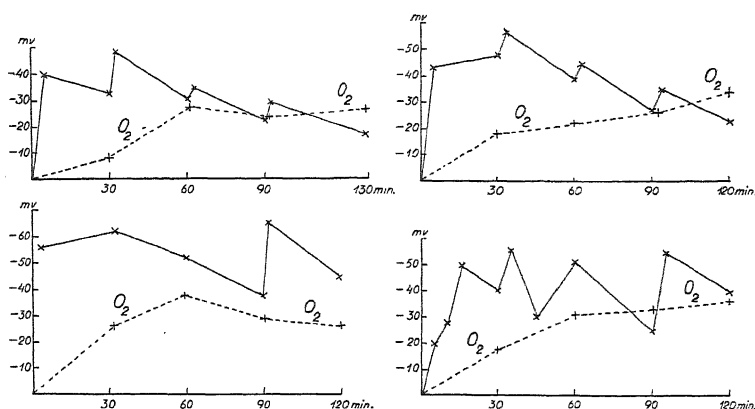


Fig. 25. Respiration (O_2 -consumption) and potentials of roots, which were held in «potential vessels». The irregularities of the potential curves refer to observations before and after the renewal of the solution. The dotted curve represents the O_2 -consumption in the intervals. As solution 0.005 m $CaCl_2$ was used.

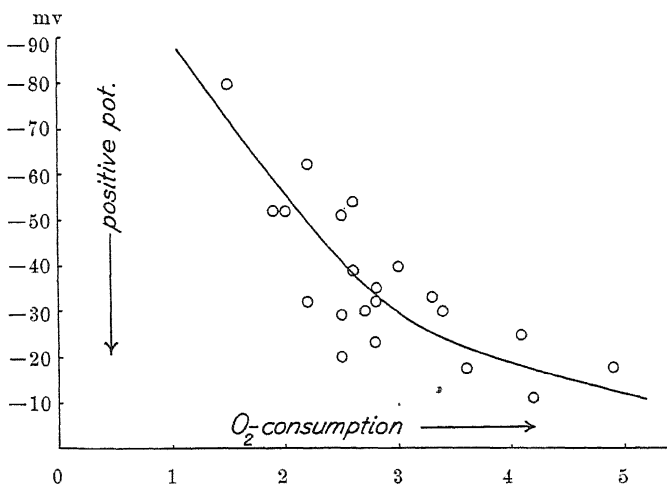
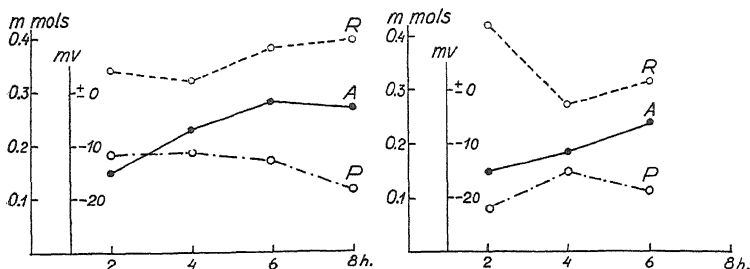


Fig. 26. A series of experiments with continuous flow of a 0.001 n KCl solution. The O_2 -consumption and the potential was determined in 30-minutes-intervals as in fig. 25. The results show clearly a relation between decreasing negative (or increasing positive) potential and respiration.

I.



II.

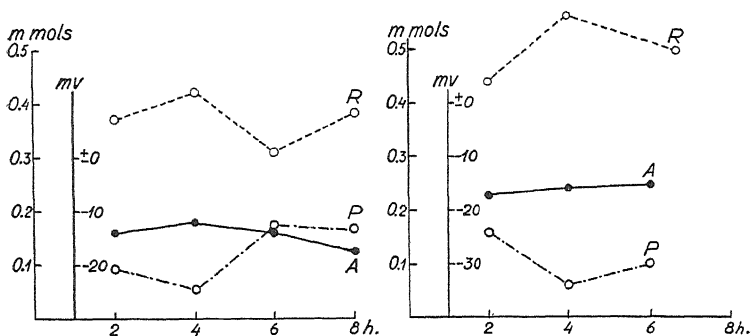


Fig. 27. In these experiments the respiration (R), root potential (P) and anion absorption (A) were determined in 2-hours-intervals. I in 0.0025 n KNO_3 and II in $0.0025\text{ n Ca(NO}_3)_2$. The relation between respiration (R) and potential (P) is obvious.

actually the case in the experiments with oxygen. Of course these experiments allow of no quite definite conclusions, because of the impossibility of following the reciprocal behaviour of respiration and potential in detail. But the results fit into the general scheme of a kind of dynamical equilibrium between potential and respiration (see also fig. 26 and 27).

The electro-chemical side of redox processes has been elucidated by several investigators (MICHAELIS 1933). Generally the redox potential is determined by the relation between the

oxidized and the reduced state of reversible equilibrium, according to the formula

$$E = E_0 + \frac{RT}{nF} \ln \frac{Ox}{Re} \quad (9)$$

Here E is the observed redox potential, E_0 is a constant, and Ox and Re are the concentrations of the oxidized and reduced states respectively of the substance. This formula holds provided that E is measured with blank platinum electrodes. A redox potential of the kind, indicated by the above formula, will not develop in a system of liquid electrodes. For this reason we cannot expect that redox processes, proceeding in the living root (-cells), will directly influence the observed boundary potentials (cf. the critical remarks by WILBRANDT, 1938, p. 251, on the ideas of LUND and ROSENE, LEUTHARDT and ZELLER, BEUTNER and LOZNER).

On the other hand a possible connection between redox processes in the cells and boundary potentials establishes our previous hypothesis that phosphate radicals are the carriers of the negative charge of the protoplasmic membrane. According to this hypothesis, which was developed on p. 275, organic molecules carrying phosphate radicals are plotted as »islands» in the surface layer at a distance of c. 1 molecule to c. 100 molecules of a non-dissociated substance per square unit. The phosphate radicals are directed towards the bulk phase and bind H-ions or metallic cations, according to their negative charge. The amount of H-ions will then be dependent on the number per square unit of the negative radical and, according to our previous experiences, also on the exchange equilibrium with metallic cations in the bulk phase. We are here primarily interested in the total number of negative valencies. If the phosphoric acid groups are involved in phosphorylation processes, connected with the aerobic respiration, a likely assumption is that an intensification of the oxidation will remove phosphoric acid groups from the surface layer. As a consequence the number of negative valencies

will decrease with increasing oxidation, and as a further consequence the negative electrical charge of the O-boundary will decrease. This is just what we have observed. Our hypothesis of phosphoric acid groups as being the carriers of the negative potential of the protoplasmic surface layer is accordingly supported also by our observations of the relation between oxygen supply and O-boundary potential.

In the present state of our knowledge of these things, we are not able to frame formulae which reflect the whole complex of the processes involved in observed relations between surface potentials and anion respiration. It is nevertheless obvious that a chain of relations exists of somewhat the following kind

$$f\left(\frac{Ox}{Re}\right) = f(\Sigma n) = f(E) \quad (10)$$

Here $\left(\frac{Ox}{Re}\right)$ is the aerobic respiration, which is connected with the anion absorption, Σn is the sum of negative valencies in the protoplasmic surface, caused by hypothetically phosphoric acid groups, and E is the observed negative surface potential. This schematical chain of processes pictures the previously described experiments with oxygene and nitrogen. Further experiments will show that special oxidative agents, such as ascorbic acid, also increase $\left(\frac{Ox}{Re}\right)$ and consequently give more positive (less negative) values for the surface layer of the root.

Some observations made by SHREDER (1939) support the theory. He found, in agreement with earlier experiments with bacteria, that vegetable cells show negative charge at cataphoresis (see also STERN, 1924, p. 24). Also animal cells show mainly negative charges. If the redox potential in red blood cells is lowered, the cataphoretic potential is also lowered.

If, now, the potential of the O-boundary is in some way related to the anion respiration mechanism, the system

will tend to an equilibrium. Provided that the equilibrium prevails at the potential E_{or} the anion respiration will be promoted by all values which are more negative than E_{or} . If this assumption is correct, the potential will not only be dependent on the oxidation power, but a change in the potential will reciprocally influence the intensity of the anion respiration mechanism.

Here we will first call attention to earlier observations, which now find their explanation. Already in the first experiments of LUNDEGÅRDH and BURSTRÖM (1933 a) we observed that distilled water gives higher respiration values than the calculated ones, or, in roots poisoned by cyanide, than observed values for the fundamental respiration. Potential measurements on roots in distilled water — containing minute traces of conducting ions — give very high values, up to 150—180 mv.

Theoretically wheat roots would give $c. 58 \cdot \log \frac{10^{-3}}{10^{-7}} = -232$ mv, but, owing to causes mentioned on p. 250 a. 267, somewhat lower values were obtained. According to equation (10) this high negative potential in the O-boundary will induce an accelerated respiration. This is perhaps an acceptable explanation of the »extra respiration» in distilled water. But other factors might perhaps intervene, because the stability of the protoplasmic membrane must be disturbed in a medium which does not contain any cations.

Among other earlier observations attention is also called to the fact that the coefficient $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ is higher in diluted solutions than in more concentrated ones. We know from the preceding pages that the ZcH -value and the E_1 -value falls with the concentration in the solution. Consequently that part of respiration, which is induced by the surface potential, would decrease at the same time as the resistance to anion absorption decreases. I shall return to these observations subsequently.

I shall now describe a series of new experiments dealing with the application of an artificial potential gradient to the roots.

If the root is linked in a short circuit by means of KCl-agar-electrodes, a current flows from the cut base (+) to the tip zone (—).

In one experiment a current of 10 volts (from a storage battery) was applied in the reversed direction of this natural current. This caused a reduced minus-voltage, possibly a +-voltage in the tip zone (always in comparison to the bulk phase). The experiment was performed in the potential vessels (see fig. 2). The root ends dipped in c. 25 cc 0.001 m KNO_3 , saturated with air. The solution was isolated by a layer of liquid paraffin. After 30 minutes the solution was renewed. In a similar vessel no current was applied to the roots. If the oxygen-consumption in the control is set = 100, the oxygen-consumption in the other vessel was only 64. The elimination of the negative charge of the roots, which in 0.001 m KNO_3 amounts to c. -70 to -80 mv, thus lowers the respiration. Similar experiments with KCl instead of KNO_3 yielded no definite results.

An experiment with 0.005 m KNO_3 was made with the application of a current of $5 \cdot 10^{-5}$ amperes (at 10 v) in the same direction as the natural current (see above). The result was a consumption of 124 O_2 , against 100 for the control. Also this result is in accord with our theory, because the added current raises the negative potential of the O-boundary.

For the further investigation of the relations between potential and respiration it is necessary to distinguish between fundamental respiration and anion respiration. Both of them are complete oxidation processes, with $\text{RQ} = 1$ at normal supply of oxygen (LUNDEGÅRDH and BURSTRÖM 1935), but apparently depend upon different enzyme systems, owing to the widely different sensitivity to cyanide (LUNDEGÅRDH and BURSTRÖM 1935) and to manganese (LUNDEGÅRDH 1939 b).

If high sensitivity to cyanide is assumed as a criterion of haemin catalysis, we can roughly characterize the fundamental respiration as a manganese catalysis and the anion respiration as an iron catalysis.

Already the positive results in KNO_3 , seen against the back-ground of the indecisive behaviour in KCl speak in favour of the assumption that most probably the anion respiration is connected with the potentials. It is a well known fact that NO_3 -ions are absorbed in much larger quantities than Cl -ions.

The electrical resistance in the circuit through the potential vessel is fairly high. It attains about 30 000 ohms with a wheat seedling, the roots of which are submerged to 50 mm. High electrical effects, attained by the application of external currents, therefore require too high voltages. 10 v generally gave a current of $5 \cdot 10^{-5}$ amperes. This current transports only 0.0015 milliequivalents per hour (1 me corresponds to 0.03 ampere-hours). In the normal anion absorption is at least 20-fold the quantity transported. The electro-chemical systems in the root which are responsible for the ion transport, must therefore work at very high voltage gradients. This seems to support the assumption of phase boundary gradients, because the transport distance is in this case very short, so that even voltages of some 50—100 mv, which are frequently measured in biological objects, might cause micro-currents of high density.

If now the anion respiration is an expression of the work done in the anion absorption, transport and accumulation, experiments like those just described cannot be expected to allow of any definite decision. Nevertheless all experiments with KNO_3 gave positive results (see table 12): an artificial increase of the negative potential in the O-boundary decreased the absorption of anions and increased the O_2 -consumption.

The difference in absorption capacity of No. 1 and 2 is due to the plants being larger and more numerous in the former

Table 12.

Respiration and anion absorption in KNO_3 at artificial increase of the negative potential in the O-boundary.

Solution Current	A Current applied		B Controls	
	NO_3 absorbed	O_2 consumed	NO_3 absorbed	O_2 consumed
1. 0.004 m $1 \cdot 10^{-4}$ (at 8 v)	0.0204 mM	0.0335 mM	0.0268 mM	0.0325 mM
2. 0.002 " $6 \cdot 10^{-4}$ (at 70 v)	0.0012 "	0.0094 "	0.0042 "	0.0084 "
3. " " $1 \cdot 10^{-4}$ (at 8 v)	0.0080 "	0.0303 "	0.0076 "	0.0270 "

case. The experiments were performed in the potential vessels (fig. 2).

We see from the experiments visualized in table 12 that, according to the theory, the absorption of anions is retarded when the negative potential is increased by the application of a current in the same direction as the natural potential gradient (+ pole of the battery linked to the lower electrode, — pole to the upper electrode of the potential vessel). But the respiration intensity is unchanged or even a little increased. This is also in accord with the theory, for an increased negative potential in the O-boundary will have a similar effect to that of placing the roots in distilled water, thus inducing a stimulation of the anion respiration mechanism without anions.

As a consequence of this the quotient $\frac{\text{O}_2}{\text{NO}_3}$ rises with the intensity of the applied current; in No. 1 it is 1.6 against 1.2 for the control and in No. 2 it amounts to 7.8 as against 2.0 for the control. The astonishingly low value of the coefficient in the controls is probably due to the fact that only the ends of the roots, which have high absorption power, were submerged in the salt solution.

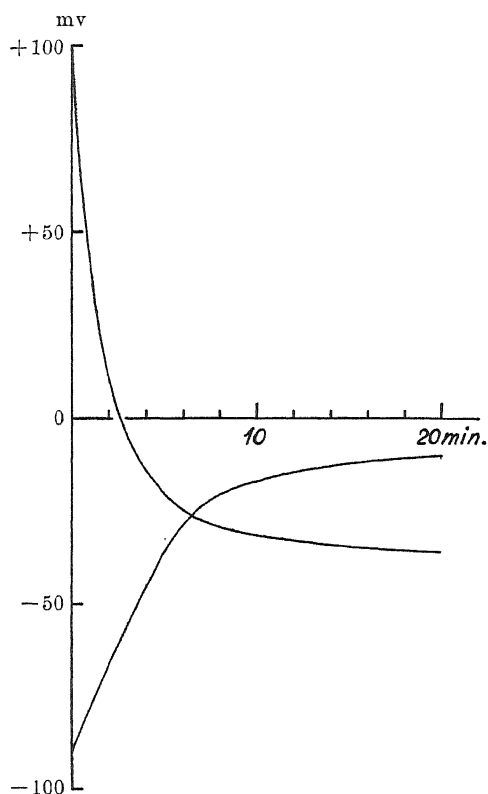


Fig. 28. Experiments with the application of a negative (upper curve) or a positive current (lower curve) to the root system. The measuring of the potentials began immediately after the opening of the circuit. See the text.

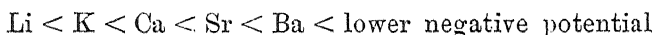
The root behaves as an accumulator, if a current is sent through it. If the current flows in a reverse direction to the normal potentials, these are also reversed. As a consequence the current is increased, by about 30 % in 30 minutes. When the external circuit is opened the root end shows positive charge, but this disappears very rapidly, so that the natural negative charge is restored within 2—3 minutes. If the added current flows in the same direction as the natural potentials, the intensity of the current decreases in the course of an hour or so. Immediately after the opening of the circuit the negative potential of the root end is very high, but in a few minutes falls to its normal value. See fig. 28.

These observations probably find their explanation in the circumstance that the biological phase boundaries are charged with metallic cations and H-ions. If the natural potential gradient at the O-boundary (and at all subsequent boundaries in the interior of the organ) is artificially increased, this increase acts as an electrolysis process, i. e. H-ions are accumulated. This abnormally high H-ion charge endures, of course, only as long as the current lasts. After the opening of the circuit the surplus is exchanged again for metallic cations, until equilibrium is attained. Observations also teach us that this restoration process is completed in about the same time as the exchange in salt solutions (about 2 minutes). See fig. 28. With the reverse direction of the current, probably an abnormal lowering of the H-ion concentration (increase of ZcOH) at the outer boundaries in the protoplasm occurs. As mentioned above, the effect of applied currents is of course a cumulative effect of all phase boundaries in the organ which also explains the high voltages obtained. The changes in the O-boundary are probably not very large, which explains why the ion absorption and anion respiration are not completely checked by a reversal of the charge of the whole root.

The experiments with artificially changed potentials in the root system have shown that a rise in the potential differences increases the respiration, but decreases the anion absorption. An artificial lowering of the potentials has the reversed effect. The changes in the respiration are comparatively small and of a magnitude which indicates that only the anion respiration mechanism is affected.

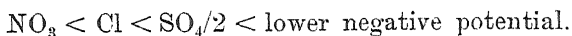
These results throw an interesting light upon the nature of the »quantitative» relations between anion absorption and respiration. The fact that both these are reversely affected by the application of artificial currents supports the conclusion we drew from a discussion of the influence of cations on the k -values and of the cause of the variation of k with the nature of the anions: The anion respiration is the

effect of a redox system, which is regulated by chemical components in the protoplasm, which, on the other hand, regulate the surface potentials. Two circumstances influence these forces in the O-boundary of the root system: firstly, the dissociation and the cation exchange in the protoplasmic membrane, and secondly, the movements of salt ions through the membrane. In distilled water we have only the first point to consider. The enduring high-potential induces a rapid oxidation. According to the theory, cyanide would check this »distilled-water-respiration», which frequently exceeds the fundamental respiration by about 30 %. But it seems difficult to effect poisoning with cyan in distilled water, so the experiment has not yet been made. The second point refers to a large number of possible ion effects. We have already mentioned the general effect of a different mobility of anions and cations: If anions A^- move faster than cations M^+ from the O-boundary to the I-boundary, this acts in the same direction as a rise in the negative potential in the I-boundary. Anions moving from the bulk phase into the protoplasmic membrane tend to diminish the amount of negative electricity in the former and to increase it (hence the negative potential) in the latter. The reverse will occur, if fast moving cations are combined with slow moving anions. In this case the cations, which move faster, tend to lower the positive potential of the bulk phase and the negative potential of the O-boundary. Potential measurements in salts with different cations and anions visualize only the exchange effect at the surface. It was found that in equinormal solutions the cations lower the negative potential in the O-boundary in the following series:



and determinations of the mobility of the ions in a membrane and in the tissue of the plant give a completely reversed series. Similarly it was found that, in equinormal solutions,

the anions lower the negative potential in the O-boundary in the following series:



The experiments with electrical currents show that also the redox system which we have called the anion respiration, really reacts to the presence of these ions in a manner which is to be expected from equations (9) and (10).

E_0 in equation (9) is the charge of the O-boundary, caused by the whole complex of native acidity (»dissociation state») and primary ion exchange. E_0 varies according to the kind of ions, as shown in the series just mentioned. E_0 acts as a barrier to anions, even if it cannot be assumed to be an absolute barrier, but only a »brake» on anion absorption. E_0 on the other hand, is an accelerator for cation absorption.

Proceeding with our working hypothesis, we can imagine the system $\left(\frac{\text{Ox}}{\text{Re}}\right)$ as built in bilaterally between two phase boundaries, the outer O-boundary and the inner I-boundary. At the O-boundary the oxidation phase predominates, causing a rise in the positive charge (= a decreased number of negative valencies), at the I-boundary the reduction phase predominates, causing reversally a fall in the positive charge (= a rise in the number of negative valencies). A number of facts speak in favour of this hypothesis: We know, as mentioned above, that the oxygen concentration falls from the O- to the I-boundary; we know that membranes or boundaries exist in the living tissue and also in the cell; and we know from potential measurements that a gradient from more negative at the O-boundary to more positive at the I-boundary exists. On the other hand, we do not know anything about the localization of the postulated boundaries beyond the assumption that the O-boundary is probably identical with the protoplasmic membrane at the surface of the organ.

Schematically the oxidation phase of the redox system which runs parallel to a decrease in Σn , acts as a mechanism for

the attracting of anions from the bulk phase in the O-boundary. But the absorbed anions again raise the negativity of the membrane, promote again the oxidation etc. in an endless chain. This is from the view-point of our theory the reason why a sort of quantitative relation exists between anion respiration and anion absorption. Under otherwise constant conditions, i. e. constancy of E_0 , it seems quite feasible to use the coefficient $k = \frac{\text{anion respiration}}{\text{anion absorption}}$. Our earlier determinations of k in series with little variation in the concentration of the bulk solution also confirm the remarkable constancy of the k -value (see LUNDEGÅRDH and BURSTRÖM 1935, e. g. table 6, p. 248, where k varies only $\pm 1\%$; LUNDEGÅRDH 1937, table 6, p. 110, differences between R_t observed (10 exp.s) and calculated varying on an average 4.8 % (k set = 2) table 7, p. 111, R_g calculated, differences for 12 exp.s on an average 13 %).

If solutions of large concentration differences are used, the k -values are generally somewhat larger in the more diluted solutions and fall with increasing concentration. This is quite in accord with the theory, because, owing to the cation exchange, $-E_0$ varies with the salt concentration. As example I submit here one curve obtained with a KNO_3 -series of tobacco. The higher absorption values here correspond to higher salt concentrations. See fig. 29.

In one series with barley the following k -values were obtained (see table 13).

If a series of experiments is performed with solutions of increasing concentration, beginning with distilled water, the negative potential is very high in the first experiments (about -150 to -180 mv in dist. water) and then falls to about -40 mv in 0.01 m (cf. fig. 5). Apart from the ion uptake, this primary membrane charge will tend to induce an accelerated respiration in the lower salt concentrations (beginning with distilled water) than in higher ones. We then get a hypothetical curve like the P-curve in fig. 30. Taking only

millimols absorbed anions

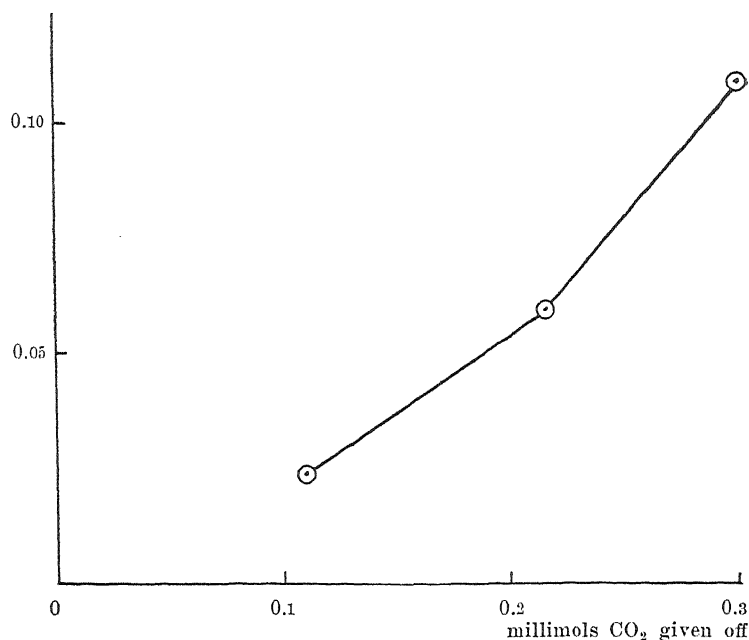


Fig. 29. The relation between absorption of anions and respiration in roots of tobacco. 0.0025 n KNO_3 .

Table 13.

Absorption and respiration experiments with barley in circulation vessels. Duration of experiments 6 hours.

Salt	Concentration	$k = \frac{\text{anion respiration}}{\text{anion absorption}}$
KNO_3	0.0025 m	1.73
"	0.0100 "	1.27
KCl	0.001 "	2.14
"	0.004 "	1.60

the transport of ions into consideration, we get hypothetically as an expression of respiration and anion accumulation, a straight line (A-curve in fig. 30). The resultant curve (AR-curve in fig. 30) will then assume a characteristic course, which, as to the upper part (higher respiration intensities) and as to the starting point (distilled water), is verified by experiments. According to this curve, the total respiration may be somewhat lower in very diluted solution of a salt than in distilled water, which was also verified by experiments.

From this analysis of the complicated form of the anion-respiration curve we must conclude that a simple mathematical formulation is not feasible. What is then left of the old expression $R_t = R_g + k \cdot A$?

We mentioned above that this expression still holds, if experiments with only small variation of the concentrations are compared. In this case the number of imported anions regulate the anion respiration and a fairly quantitative relation, expressed in the coefficient k , prevails. The expression $R_t = R_g + k \cdot A$ is nevertheless only an ideal limit, and its realization presumes not only limited variations in the concentrations, but also constancy in respect of cations. Changing of cations always influences the potentials more or less and also the k -values (LUNDEGÅRDH 1937).

If the relation $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ is taken as an expression of the work done in raising the anions from the O-level to the I-level (see p. 319) k must also depend upon the quotient $\frac{c_i}{c_o}$. Consequently a rise in c_o (= concentration of the salt in the bulk phase) will tend to diminish k . The hypothetical A-curve in fig. 30 ought for this reason to bend upwards at the higher concentrations. For the sake of comparison, some results of the analysis of the expressed sap of roots and bleeding sap from the cut end of roots are collected. The analysis was made spectrographically in this laboratory. Compared with the concentrations in normal nutrient

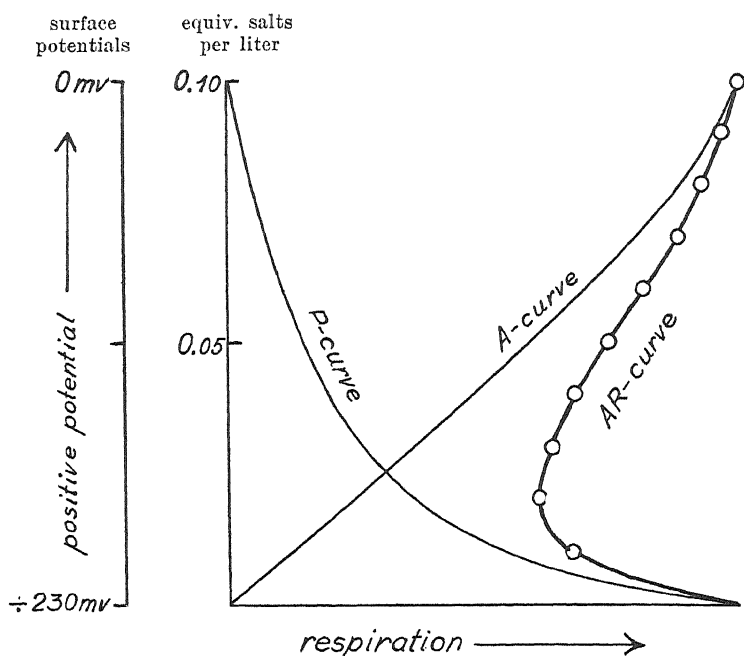


Fig. 30. Diagram of the factors which are assumed to participate in the anion respiration. The *A*-curve represents the approximate proportionality between the amount of accumulated anions (*A*) and the anion respiration (*k.A*). This curve generally rises with the concentration of salts in the solution. Owing to the cation exchange the concentration of neutral salts on the other hand determines the surface potential of the roots (see the two scales on the ordinata). The relation between surface potential and respiration is visualized by the *P*-curve. As a resultant of the *A*- and the *P*-curves the *AR*-curve represents the relations between absorption and anion respiration.

solutions used in this laboratory, the root performs positive accumulation work, especially in respect of potassium, magnesium and manganese. It is interesting to notice that the bleeding sap is more diluted than the expressed sap, which seems to indicate that the maximum accumulation work is done somewhere on the way between the bulk phase and the central vessels in the root. If the concentration of potassium in the bulk phase is suddenly increased up to 0.08—0.05

Table 14.

Concentration of some cations in the root. All figures are in millimols/liter.

	K	Ca	Mg	Mn
Expressed sap . .	28—49	1.7—2.8	4	0.6—1.2
Bleeding sap . .	7—28	0.3—0.7	0.1—1.6	—
Nutrient solution	1.9—2.4	1.5	0.5	—

mmols, equimolarity between solution and root tissue will be attained. But, as mentioned on p. 306, this will very probably not mean an elimination of the accumulation mechanism, because the latter is also an acceleration mechanism, which probably works positively even with hypertonical bulk solutions. Otherwise the expressed sap gives only an average of the cation concentrations. The ions are probably far more concentrated at the active surfaces than in the mass of the cells or tissues. But the work performed in accumulation will theoretically nevertheless be somewhat less at high outer concentrations. An elimination of the accumulation mechanism is improbable also for that reason, because ions, not salt molecules, are absorbed. The exchange and transport of ions will always require special mechanisms.

The new conception of the theory of salt absorption and respiration does not vitiate one of the chief conclusions of our earlier work, the existence of two different types of respiration, 1. anion respiration and 2. fundamental respiration. The remarkable insensitiveness of the latter to a number of influences which control the salt respiration, have been repeatedly mentioned.

In this paper we have not dealt with the anaerobic anion respiration, which was discovered in the work of LUNDEGÅRDH and BURSTRÖM (1935). The aerobic process predominates in the natural life of the plant. Anaerobic respiration

seems to be a relief, if the aerobic life of the roots is temporarily checked, but it is probably soon stopped, as hydrogen acceptors for complete carbohydrate decomposition are accessible only in a very restricted amount. The polemics of HOAGLAND and STEWARD against the work of LUNDEGÅRDH and BURSTRÖM is therefore quite ineffective also in respect of the anaerobic anion respiration. These investigators have apparently not been able to measure the small amount of anions, which really are absorbed during the first hours of anaerobic life, and LUNDEGÅRDH and BURSTRÖM have never pretended to assume that this limited power of anaerobic anion absorption is a process of any importance in the normal life of the plant. Nevertheless it is very interesting, from a theoretical point of view, to see that in the living tissue still other redox systems exist than the two dominant ones, which we have already discussed.

In order to throw light on my opinion as to the anaerobical uptake of anions, I have collected in table 15 the results of a number of old and new experiments. In all these experiments the uptake of cations have also been determined, but the figures are omitted here, since they are not significant for the process.

The experiments show clearly that even a diminished O_2 -supply considerably retards the anion-process (Nos. 1—4). A complete exclusion of oxygen checks completely the absorption of chloride (Nos. 7, 8, 9—12), but allows measurable quantities of nitrate to be absorbed. This fact is probably due to the oxygen supply brought about by the nitrate ions. We know that the fundamental respiration is capable of absorbing the slightest traces of oxygen. Even under quite anaerobical conditions (in nitrogen without circulation) small traces of oxygen might come in or are perhaps stored in the root system at the commencement. These traces are perhaps sufficient to maintain a small reduction of NO_3 (this process is coupled with the fundamental aerobical respiration).

Table 15.

Absorption of anions with different supplies of oxygen or in pure nitrogen. Experiments 1—4 were made in 600 cc non-circulated solution, Nos. 5—12 were made in circulated solution. Duration of experiments in Nos. 1—4 20 hours, in Nos. 5—12 6 hours. 15 seedlings, 2—3 weeks old, and grown up in photo-thermostats, were used in each experiment. All experiments were duplicated.

No.	Oxygen supply	Salt	Absorbed anions	Reference
1.	1.84 mmols/lit.	0.00125 n KNO_3	0.124 me NO_3	Lundegårdh a. Burström 1935
2.	0.19 »	» » »	0.070 » »	» » »
3.	0.79 »	» » KCl	0.064 » Cl	» » »
4.	0.27 »	» » »	0.025 » »	» » »
5.	circul. air	0.002 n KNO_3	0.410 » NO_3	Lundegårdh 1937
6.	nitrogen	» » »	0.126 » »	» »
7.	circul. air	» » KCl	0.071 » Cl	» »
8.	nitrogen	» » »	0.006 » »	» »
9.	circul. air	» » »	0.081 » »	New experiments
10.	» nitrogen	» » »	0.000 » »	» »
11.	» air	0.001 » »	0.070 » »	» »
12.	nitrogen	» » »	0.004 » »	» »

The objections advanced by STEWARD and HOAGLAND (several papers) against the statements of LUNDEGÅRDH and BURSTRÖM as regards certain anaerobical anion respiration processes, is misleading and based on an incomplete analysis of the elementary processes involved. It is a fact that with a restricted O_2 -supply certain processes are brought in, which are much more sensitive to KCN than any aerobical process, and which to some extent promote the uptake of nitrate. These processes certainly ought to be studied more in detail, but they definitely ought not to be confused with the aerobical anion respiration, which dominates the normal absorption of salts. Everyone who has worked with roots of higher plants knows

that these are, as a rule, very sensitive to a prolonged stay under anaerobical conditions. As an indication of the disturbed metabolism, organic acids are frequently given off from such roots. These facts show, more than any others, that the anion respiration mechanism is out of order. Further, anaerobic bacteria readily invade the experimental vessels, if care is not taken to work under aseptic conditions.

Returning to the dominant aerobic processes, I am going, finally, to describe some experiments with artificial increase of the oxydation power of the root surface. I mention first one experiment with an oxidizing agent, ascorbic acid in very diluted concentration. Root systems were placed for two hours in distilled water + about 1 mg ascorbic acid in 1 liter. After that time the root potentials were determined in 10^{-4} m HCl. For the sake of comparison, similar plant groups were placed in 0.002 m K-phosphate and 0.002 m KCl. See table 16.

Table 16.

Potentials of root ends after treatment in different solutions during 2 hours.

Treatment	Root potentials in 10^{-4} m HCl
0.002 m K-Phosphate	-82 mv
0.002 m KCl	-67 mv
0.1 mg ascorbic acid/liter aqua	-48 mv

The experiments show that ascorbic acid has a marked lowering effect on the negative potential of the root. This is in accord with the oxidation qualities of this acid, for an increase in the oxidation power according to formula (10) will raise the positive potential of the protoplasmic surface. Being an acid the ascorbic acid ought to change the ZcH value a little (p. 298), but owing to the dilution (c. 10^{-5} mols/lit.) this effect is negligible.

In other experiments the influence of ascorbic acid on the ion absorption was studied. In very small concentrations (0.1 mg/lit) the anion absorption was somewhat increased (c. 10 %), a result which is in accord with the potential measurements of table 16. The respiration was somewhat reduced in 6 hours, which is also in accord with the lower negative potential in the O-boundary. Cation absorption was unaffected. In higher concentrations the ascorbic acid has detrimental effects on the growth (the root ends are finally bent sideways), and the ion absorption is also checked. See table 17.

Table 17.

Experiments with ascorbic acid. Basic solution 0.002 m KCl. Circulation vessels, streaming air. Duration 6 hours.

Ascorbic acid added	Cl absorbed	K absorbed	Total respiration
0.1 mg/liter	0.059 millimols	0.067 millimols	0.798 millimols
1.0 "	0.040 "	0.016 "	0.844 "
10.0 "	0.010 "	K emitted	1.181 "

In all experiments in table 17 controls without ascorbic acid were made. The rise in respiration corresponds to the increased total respiration in diluted acids (see LUNDEGÅRDH 1937) and is probably partly due to autolytic processes. In the highest ascorbic acid concentration, too, the acid nature appears (about 10^{-4} mol). As a consequence, cations are removed from the root. The stimulated respiration might possibly be caused by the oxydation power of the acid, even in the toxic concentrations. But it is significative that mineral acids also cause a violent rise in respiration intensity. This is not in accord with the primary potentials, because these are lowered in pure acids, but is perhaps due to the disorganizing effect (cf. p. 246).

Special observations on roots submerged for some time in diluted mineral acids, show that this causes a lowering of the

potentials below the primary values, which illustrates the assumed disorganizing effect on the protoplasmic membrane. We have demonstrated this effect in the time series in fig. 12. Several other experiments, which are not here related in detail, support this view. Also in strong organic acids, e.g. indolyl acetic acid, a lowering of the potential of the root is observed. Pure acids thus act in the same general manner as potassium salts (p. 290), but more intensively. A biological antipode is calcium, which »conserves» the membrane, so that the negative potential endures. Manganese, too, has a »conserving» effect (see LUNDEGÅRDH 1939). As a secondary effect of the pure acids then appears the abnormal stimulation of the »fundamental respiration» and the induction of abnormal oxidation processes in injured cells respectively.

We have seen that an oxidation agent, e.g. very diluted ascorbic acid, causes an expected decrease of the potential of the root. Another way of increasing the oxidation intensity at the root surface is to supply respiration material from outside. In table 18 some experiments are recorded dealing with the

Table 18.

Potentials of roots from plants held in solutions of glycerol, mannite and glucose. The solutions were aerated with pure oxygen.

No.	Bulk phase	Duration of treatment	Potential of root ends (20 mm, total length of cut roots 60—70 mm). In No. 4—5 whole plants.	
			in 0.001 m KCl	in 10 ⁻⁴ m HCl
1.	0.02 m glycerol	2 hours	—	—53 mv
2.	0.02 m mannite	2 »	—	—54 »
3.	0.02 m glucose	2 »	—	—28 »
4.	0.02 m mannite	1 »	—86 mv	—92 »
5.	0.02 m glucose	1½ »	—60 »	—64 »
6.	0.02 m mannite	2 »	—77 »	—55 »
7.	0.02 m glucose	2 »	—73 »	—46 »

addition of different carbon compounds to the bulk phase. The intact plants were used and roots then cut off for potential measurement.

The table contains determinations both of whole plants, observed in the potential vessels (fig. 2), and of roots cut from the treated plants and observed in the apparatus fig. 1. The series Nos. 1—3, 4—5 and 6—7 are concordant in respect of the general results. Only glucose, which is apt to serve as respiration material, lowers the potential. Mannite and also glycerol, which are not combusted by higher plants, have no effect on the potential. The plants which were used for the experiments in table 18 contained less glucose than those which were used for the experiments of table 11. Table 19 shows that, in the former plants, not only the fundamental respiration but also the anion respiration was stimulated.

Table 19.

Experiments in circulation vessels and streaming air. Duration 6 hours. Plants about 3 weeks old. In all experiments small amounts of Ca and traces of Mn were given off from the roots. Temperature 24° C. All figures calculated in millimols per 1 g dry weight of roots.

Salt	A. Plain salt solution		B. 0.003 m glucose added		
	Anions abs.	Total CO ₂	Cations abs.	Anions abs.	Total CO ₂
0.002m KCl	0.065 Cl	0.562	0.127 K	0.099 Cl	0.677
» » KBr	0.079 Br	0.570	0.180 »	0.099 Br	0.675
» » KJ	0.052 J	0.615	0.082 » ²	0.082 J	0.878
» » KHCO ₃	—	0.513	0.120 »	—	0.825

Table 19 shows that in this case (cf. p. 328) the anion absorption increases with the supply of glucose. The increase for Cl and J is more than 50 % of the quantity absorbed without glucose. This is in accord with the increase in total respiration. On the other hand, this stimulation of the total

respiration is no doubt not restricted to the anion respiration, but also includes other oxidation processes. We observe this fact in the experiment with KHCO_3 , which shows an even larger increase in total respiration than the experiments with halogene salts. Much more glucose is taken up by the root than is used for the increased respiration. Quantities of about 0.2 mmols of glucose absorbed correspond to $6 \times 0.2 = 1.2$ mmols CO_2 , which is more than the whole total respiration and four to twenty times more than the increase in respiration brought about by the glucose supply. Even if we admit that a part of the glucose is used up by bacteria at the root surface and another part consumed in stimulated growth, we must conclude that the root takes up large quantities of glucose, which are not completely combusted to CO_2 . Analysis of the expressed sap of roots shows that the acidity is somewhat increased, if the roots are fed with glucose.

12. General discussion of the results.

The present paper is an attempt to give an account of the results of eight years of experimenting in the mechanism of ion absorption in plant roots. A number of new experiments are also included, which, on different points, support the theoretical discussions. It is a difficult task, for all attempts to apply chemical-physical view-points to biological processes leave behind them several obscure questions. On the other hand, the complicated nature of the biological processes ought not to discourage us from attempting to apply physical-chemical principles. The complications are due to the multitude of the structural bases of life processes (cf. FREY-WYSSLING 1938). Structure and interfaces play an important rôle in the living cell. The cause of the fact that a complete combustion of sugar by means of enzyme systems has not yet been effected *in vitro* is surely the circumstance that the chain of chemical reactions in the cell is distributed over a structural system which regulates the intimate contact between the intermediate

products of the reactions. The alcoholic fermentation also seems to postulate a »living» structure for its normal course (cf. OHLE, 1931).

In respect of the uptake and accumulation of ions, which is one of the fundamental processes of the living cell, the researches performed at this institute fully confirm the importance of structure. We have already referred to BURSTRÖM's (1939 b) observations on the cessation of ion absorption in crushed roots. The fact that ion absorption is clearly related partly to surface phenomena (potentials), partly to oxidation processes which are in some way »polarized», shows that here a typical case of structural interaction in chemical processes occurs. The conception »structure» is not here intended to be synonymous with »living organization». I believe that the »living structure» is only a special, though extensive, chapter of »chemical structure». The stoichiometry of the proteins etc. is very complicated and renders possible couplings and bindings between molecules which do not exist in classical inorganic chemistry. In addition, the special conditions on surfaces and interfaces tremendously augment the interaction of chemical and physical processes.

The experimental basis for my conception of a theory of ion absorption and accumulation has been extended during recent years. This is the reason why the outlines of the theory have changed somewhat. A »working theory» or a »working hypothesis» is only a necessary aid to continued research work. It fulfills our aim to look upon observed processes as the result of a mechanism which is built up within the living organism, and which it must be possible to analyse from a physico-chemical point of view. But everywhere we are only at the beginning, and our theories are always only provisional. Their chief purpose will be to stimulate further research work. My theory of anion respiration, which was developed in papers from 1933 to 1935, has nevertheless still retained the original principle that *a certain quantitative relation exists between salt*

absorption in living cells and organs and respiration. This is the first principle of the theory.

The second principle is *the fundamental difference in the mechanism of cation absorption and anion absorption.* This principle necessarily does not include the general postulate that absorption of ions always starts as an exchange of ions at the surface of the protoplasm (p. 269). But the fundamental difference really appears in the fact that *the exchange of a cation must occur at a different place from that of the exchange of an anion.* Extensive series of experiments support this second principle of distinct mechanisms for cation and anion absorption. In these experiments absorbed and exuded ions have been carefully determined and parallel measurements of the respiration have been made. The principle of the different absorption mechanisms is indirectly supported by potential measurements, which show that, generally, the ion absorption is not a simple diffusion process, but, as mentioned above, an exchange process at the surface of the protoplasm.

The third principle of the theory is that the anion respiration is a process, which differs biochemically from other respiration processes (= fundamental respiration) within the cells. The anion respiration is rather sensitive to cyanide (LUNDEGÅRDH and BURSTRÖM 1935), whereas the fundamental respiration is very resistant in this respect and also to certain metallic poisons which affect the anion respiration. Furthermore the choking of the oxygen supply checked the anion absorption mechanism already at an O_2 -tension which enabled as much as 20—30 % of the fundamental respiration to proceed. Finally new researches have contributed to elucidating the biochemical characteristics of the two respiration groups: the fundamental respiration is partly a manganese catalysis, whereas the anion respiration is not influenced by this metal. The high sensitivity of the latter to cyanide speaks in favour of the assumption that iron is the catalytical agent for this process (at least according to

WARBURG's views). Now the redox potential of $\frac{\text{Mn}^{+++}}{\text{Mn}^{++}}$ is very high (-1.5 volts) in comparison to $\frac{\text{Fe}^{+++}}{\text{Fe}^{++}}$ (-0.74 volts). This is perhaps the cause of the fact that the fundamental respiration is checked only at very low oxygen concentrations.

The fundamental respiration is certainly the sum of several aerobic reactions, because some respiration activity is still left in roots which are free from manganese. On the other hand, the normally aerobic anion respiration seems not to be the only process involved in the anion absorption. Also in the absence of oxygen a reduced ion absorption occurs, accompanied by anaerobic anion respiration (LUNDGÅRDH and BURSTRÖM 1935), which seems to be biochemically different from the aerobic process. This complexity of the metabolism does not detract from the importance of the proposition that there are at least *two groups of aerobic respiration processes, which dominate the normal aerobic life of the cells.*

The fundamental aerobic respiration in wheat roots implies — apart from the aerobic anion respiration — a complete combustion of glucose ($\frac{\text{CO}_2}{\text{O}_2} = 1$). A super-normal supply of glucose from outside seems, on the other hand, also to cause incomplete combustions (see p. 358). The probable coupling between respiration and growth shows that quantities of glucose are drawn sideways into processes of very low energy gradient.

Absorption and accumulation of ions involves osmotic work, which is expressed in the formula

$$A = R T \cdot \ln \frac{c_1}{c_0} \quad (\text{cf. p. 319}).$$

If the salt $M^+ A^-$ is accumulated, the work to be done might as well be directed only upon one of the ions, e. g. the anion.¹ The anion respiration furnishes the accumulation

¹ Theoretically it is quite possible that A might be related to the cation absorption. Hitherto no case of »cation respiration» has been observed,

energy A (p. 320). Starting from observed values of the relation $\frac{c_{\text{glucose}}}{c_{\text{anions}}}$, it is found that the actual energy which is liberated exceeds the quantity A in the above formula.

It is now to be expected that A is not only used for actual accumulation from the O-level to the I-level, against the gradient $c_1 \rightarrow c_0$, but also for the acceleration of diffusion work, which proceeds in the direction of the gradient. Several authors (MASON and PHILLIS 1937, CRAFTS 1939, a. o.) have emphasized the rapid movement of solutes, including ions, through the plant. The fact, which is revealed by my own researches, that ions probably do not pass the protoplasmic membranes by diffusion, but by ion exchange reactions, also speaks in favour of the assumption that diffusion plays a subordinate rôle as a motoric agent. For this reason it seems probable that the mechanism for ion absorption and accumulation is also put in action if ions are to be transported in the direction of a diffusion gradient, e.g. in absorption from a rather concentrated nutrient solution or in the translocation of salts from the accumulation zones in the roots to other parts in the plant. If this conception is sound, no outstanding difference exists between accumulation and exudation of ions. In the former case, the movement proceeds in the direction $c_0 \rightarrow c_1$, in the latter case in the direction $c_1 \rightarrow c_0$. The quantity of energy involved in the process will then be used primarily for overcoming the resistance in the membranes, hence, in accordance with the scheme of fig. 31, the gradient $c_0 \rightarrow c_a$ (by accumulation) or $c_1 \rightarrow c_a$ (by exudation). The levelling between c_a and c_1 or c_a and c_0 (see fig. 31) might then be thought to occur in the direction of the diffusion gradient

in the sense that a special energy-spending process is coupled with the entrance of cations into the cells or organs. If acids are produced in the metabolism, and as a consequence a one-sided cation absorption is promoted (p. 305), this ought not to be confused with a true cation respiration, because the mechanism of entrance of cations in that case is the adsorption potential.

by convection or »directed diffusion» along molecular chains. In accumulation it can be postulated that $c_a = c_1$, but in exudation c_a is always larger than c_1 . The difference between accumulation and exudation is then limited to the direction of the passage between the levels O and I. It seems to be of little use again to introduce the term »adenoid» (OVERTON) in order to indicate the translocation of a solute by means of energy-spending processes (see COLLANDER and HOLMSTRÖM 1937, who propose this term; FREY-WYSSLING 1938), the more so as also certain presumed »permeability» phenomena have revealed themselves as energy-requiring processes (ARISZ, 1939).

According to the formula $A = RT \cdot \ln \frac{c_1}{c_0}$ osmotic work is performed in the accumulation. We meet here the proximate relation between accumulation of ions and root pressure. It is not astonishing that a root pressure of several atmospheres has been measured in isolated root tips (WHITE 1938). If salts are accumulated up to the concentration c_1 within a cell or a group of cells, the hydrostatic pressure

$$\pi = c_1 \cdot RT$$

arises at equilibrium. This is the pressure within the cell, which cannot be directly utilized for the root pressure, which is measured on the bleeding sap. The pressure of this sap refers to the free moving water in the vessels. The interpretation of the root pressure as an osmotic pressure, which arises from accumulation work, postulates an excretion of salts into the vessels. The fact that the bleeding sap always contains considerable quantities of mineral salts (see p. 351; also SABININ 1931, LAINE 1934) proves that a real exudation takes place. One can imagine the state of affairs in the root, in conformity with fig. 32, as a zonal accumulation and excretion. New analyses, carried out in this laboratory, showed a concentration of 0.030 mols/liter (total ion content) of the bleeding sap at a concentration in the nutrient solution of 0.005 mols/lit.

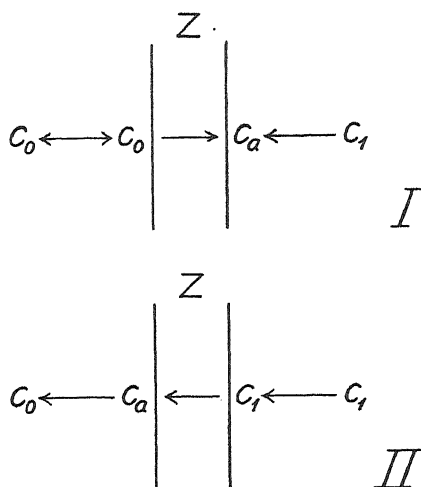


Fig. 31. Scheme of accumulation (I) and excretion (II) in a living organ Z. See the text.

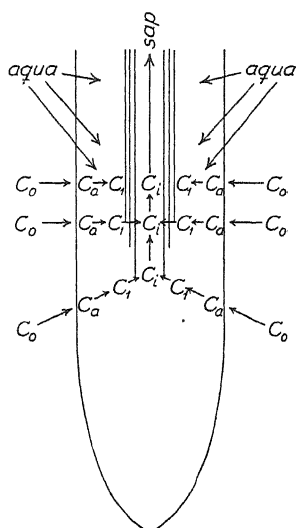
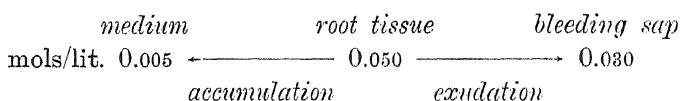


Fig. 32. Scheme of the accumulation process within a root.

At the same time the expressed sap of the roots showed a concentration of 0.050 mols/lit. or more. We get then the following scheme:



The osmotic pressure of a 0.03 mols/lit. salt solution is c. 1.3 atmospheres. But this pressure refers to the upper part of the aqueous pillar in the vessels. If the salts are given off chiefly in the basic parts of the vascular system, the concentration, and consequently also the osmotic pressure, will be larger here. According to SIERP and BREWIG (1935) the water is primarily absorbed in the zone of root hairs, hence in the same region as the absorption of salts, according to BURSTRÖM (see also GREGORY and WOODFORD 1939). The osmotic suction will therefore be considerably larger in this

region than in the upper parts of the vascular system, and magnitudes of the degree mentioned by WHITE (1938) will appear quite feasible. Normally the pressure of the bleeding sap is associated with »suction forces» from the transpiring leaves.

SPEIDEL (1939) recently found a quantitative relation between respiration and bleeding, and HENDERSON (1934) describes a parallelism between water absorption and root respiration. I imagine that the connecting link between respiration and water absorption is the anion respiration, which, by means of the mechanism that we have already analyzed, regulates the osmotic pressure in the lower ends of the vessels. If the oxygen supply to the roots is checked, the anion respiration is practically checked too, and a number of observations show that also the exudation of bleeding sap stops.

The existence of an accumulation and an exudation of salts postulates an assymetry or a polarity of the »living» layer which performs the osmotic work. This is the reason why we have distinguished between an O-level and an I-level. Permeability investigators have discussed the terms »intrability» and »permeability» (HÖFLER 1934). The former conception would mean the passage of a substance through the outer protoplasmic membrane of a cell. Permeability, according to HÖFLER, would mean a passage also through the membrane of the vacuoles (see also BROOKS 1938). It seems to be a common opinion among permeability investigators (cf. FREY-WYSSLING 1938, p. 154) that the membranes of the vacuoles are of a »tighter» structure, i. e. less permeable than the surface membrane. But it seems to me that these distinctions are not supported by facts of a more general bearing. In large cells of certain algae the protoplasmic layer is very thin; on the other hand meristematic cells have no common sap space. In respect of the accumulation in a tissue, a »symplast», the vacuoles in the single cells probably do not play any specially important rôle in the whole mechanism of accumulation. Potential measurements under systematically

varied conditions lead to the conclusion that the protoplasmic surface consists of one or a few layers of oriented molecules (DANIELLI 1936), of which the majority are either undissociated or have their valencies protected («screened») by connections with neighbouring organic molecules. Calculating with the actual cH of the protoplasmic surface and the known diameters of phosphatides and similar surface-active substances, one can draw the conclusion that only c. 1 % of the molecules in the protoplasmic surface extend negative valencies against the bulk phase. At normal LcH-values (c. 10^{-4} to 10^{-6}), the relative number of positive valencies is probably of the magnitude 0.01 % of the total number of molecules in the surface (p. 271).

The assumption that the protoplasmic surface forms a mosaic of different types of molecules, of which the majority are undissociated or «screened», and some of positive, some of negative charge, is based on my own experiments and known experience of molecular structure and monolayers. The charged molecules probably constitute isolated groups, »islands», in undissociated surroundings (see fig. 33).

The predominating negative charge of the protoplasm — which seems to be a general characteristic of it (p. 338) — ensures a strong adsorption of metallic cations, even in very diluted solutions (see p. 258). While the interior of the protoplasm, too, usually has an acid reaction (see PFEIFFER 1929, FREY-WYSSLING 1938, p. 127), metallic cations will probably invade the bulk of the protoplasm. The importance of this fact for the consistency, stability etc. of the protoplasm is frequently discussed in the literature. For the theory of ion absorption, the importance of this circumstance lies in the fact that the cations of the nutrient salts are always accessible within the protoplasm for the maintaining of a proper balance with anions which are formed in the protoplasm or are introduced into it from the outside, through anion respiration.

The starting point for the researches into the relation between the surface charge of the protoplasm and the anion

respiration was the establishment of a »native charge», called ZcH_{\max} , which reflects the acid dissociation of the membrane. This ZcH_{\max} causes a very high charge in distilled water (150—200 mv). In solutions which contain metallic cations, the charge is considerably lower, but even in complete nutrient solutions usually attains -50 to -60 mv. A charge of this magnitude is also significant for isolated large cells of algae (BLINKS 1935, JACQUES 1939). Such a negative charge must retard the absorption of anions. From experiments with

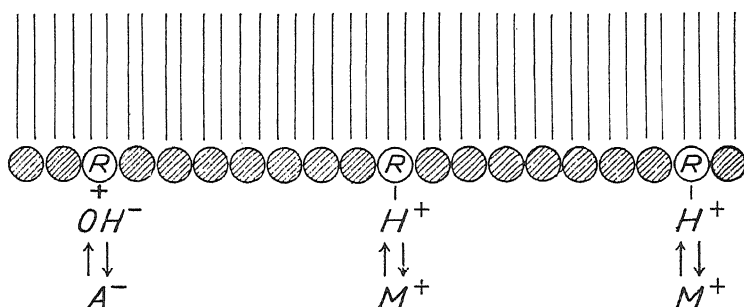
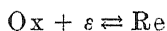


Fig. 33. Scheme of the protoplasmic surface. The hatched circles are the non-dissociated molecules. R are the positive, or negative »heads» of the dissociated molecules, which participate in the absorption of anions (A^-) or cations (M^+) of neutral salts.

varied oxygen supply and varied salt concentration — and consequently varied surface charge of the roots — the elements of an electro-chemical theory for ion accumulation was deduced.

Originally the idea of a reversible electron equilibrium, according to the formula



(see MICHAELIS 1933, p. 25, 26) served as the back-ground for the theory (LUNDEGÅRDH 1939 a).

It seems to be quite feasible to claim the existence of a redox system, the equilibrium of which depends, among other factors, upon the oxygen pressure, and that the anion res-

piration is such a system. It is no use indulging in vain speculations as to the organization of such a complex of reactive substances (enzymes + donators + acceptors etc. + glucose), by means of which a complete oxidation of glucose ($RQ = 1$) is obtained, but one can, *a priori*, postulate that the intensity of the oxidation phase is maximal at the free surface of the organ, where the oxygen pressure is maximal.

If, therefore, $\frac{O_x}{R_e} = O_{or}$ at the surface, then, in the interior of the organ, $O_{or} > I_{or}$, if I_{or} is the symbol of the oxidation state in the interior. The surface and the interior of the organ thus form an electric element with the electromotorical force

$$E = \frac{RT}{nF} [\ln(O_{or}) - \ln(I_{or})]$$

Because the living protoplasm lacks surfaces which can catch electrons in the same way as blank platinum electrodes, without immediately changing the charge into chemical energy, it is impossible to imagine a direct influence from such a system on the surface potential of the protoplasm (cf. p. 337). The redox system, on the other hand, will perform chemical work, e. g. transport of ions against a diffusion gradient. And one of the tasks of the anion respiration seems just to be the transport of anions from the O-level to the I-level. It may be indicated as a fact of considerable importance that a close relation between the oxidation intensity and the magnitude of the potential difference in the interface protoplasm : bulk phase exists. I have tried to arrive at a possible explanation of this fact: if the negative potential is caused by negative valencies of molecules, which are in some way links in a chain of oxidation reactions, an increase in the oxidation intensity would reduce the number of these molecules, and consequently the negative potential.

The electro-chemical theory of salt absorption is visualized in the diagram fig. 34. The long rods represent the organic molecules which carry the negative and positive valencies

respectively. In reality the »charged» molecules are separated by many »uncharged» molecules, and the negative ones far exceed the positive ones.

The charged molecular rods are thought to be coupled together end to end in a way similar to that generally admitted for molecules of lecithin or fatty acids (cf. BUNGENBERG DE JONG & BONNER 1935, LANGMUIR 1939, p. 22). A similar coupling in chains is claimed to be characteristic of the main substance of protoplasm, according to FREY-WYSSLING (1938). In the schematic figure it is assumed that this coupling in chains is extended continuously from the O-level to the I-level. This is certainly not the case in a »sympplast» and scarcely even in a »monoplast». Solid couplings between molecular rods cannot exist, owing to the streaming in the protoplasm. On the other hand, a kind of kinetic coupling, i. e. a transitory connection between long molecules, e. g. protein molecules, is quite feasible. These flexible molecular chains are probably of importance for the transport of ions, because they carry charged groups (e. g. COOH or NH₂), which are screened by inorganic ions. In this way ions may move very fast from one side of the protoplasm to the other, simply by jumping along such a molecular chain, as suggested in fig. 34. This is the same type of transport as that along a monolayer, which has been studied by LANGMUIR (1939), ADAM (1930) and others. Even if the molecular chains are not at every moment completely continuous, the mere existence of such »running-tracks» for ions will enormously promote translocation within cells and organs.

In fig. 34 the hypothetical redox-system, which controls the anion respiration, is schematically designed as a solution element. The chemical activity of this element would appear as an elimination of negative elementary charges in the O-level, and reciprocally the elimination of elementary positive charges in the I-level. Such a redox-element may in reality consist of chains of molecules or groups of molecules, along which an electron wave is forwarded. It is well known that

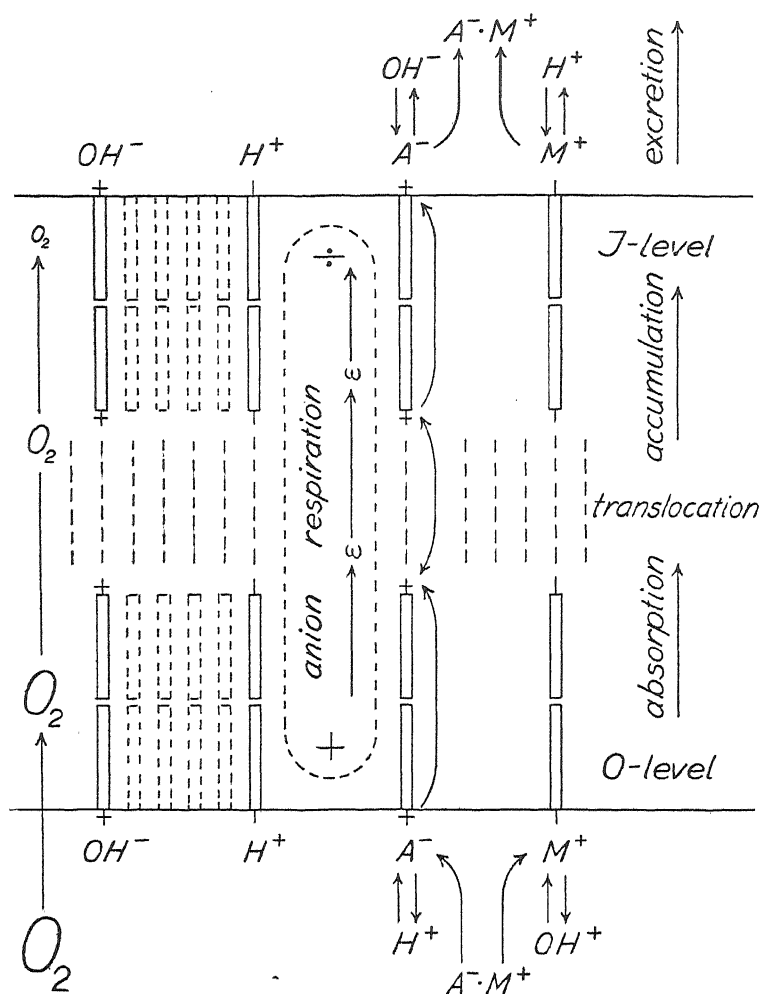


Fig. 34. Scheme of the electro-chemical theory of absorption and accumulation of ions. Further details in the text.

haemin iron, in the shape of cytochrom (KEILIN 1925, YAKUSHIJI 1935) or »respiration ferment» (WARBURG), has been traced everywhere in vegetable cells, and in roots also. I have myself repeatedly proved the presence of an absorption spectrum of

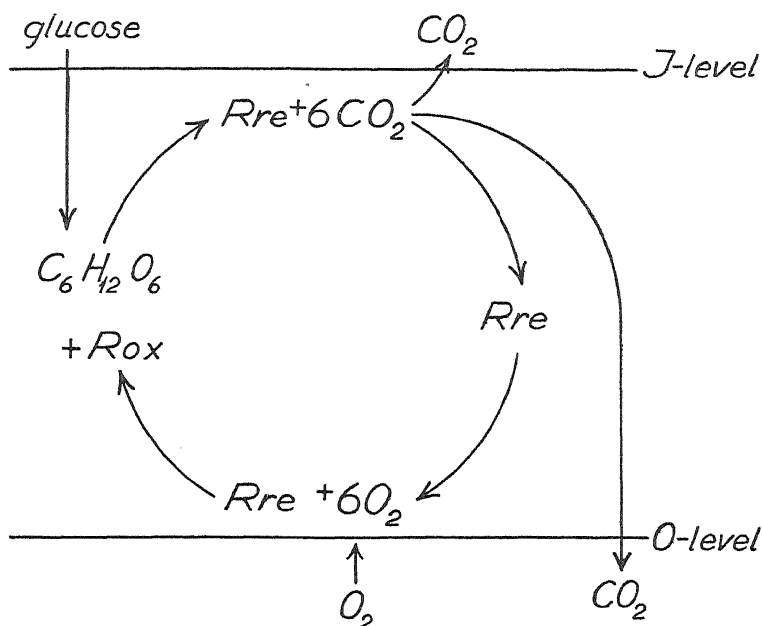


Fig. 35. Scheme of the anion respiration. Rre and Rox the reduced or oxidized stage of a hypothetical redox system, which controls the surface potential and the absorption of anions.

a type similar to cytochrom in living wheat roots. It seems possible, then, that a redox system is built in addition to such haemin molecules, even if these molecules, as the Japanese school thinks (SHIBATA, TAMIYA), only function in oxygenation processes. The driving force of this system is glucose, which is normally supplied to the root cells from the leaves through the vascular system, together with oxygen, which is supplied from outside (see fig. 35). Reciprocally the molecular hydrogen (rH according to CLARK) decreases from the inside of the organ to the outside, because the equilibrium in the redox-system is displaced to the Re -side at the I -level. The circle of cation absorption is outlined in fig. 36.

The hypothetical redox-system (fig. 35) is thought to work along an O_2 -gradient and can therefore be conceived as being

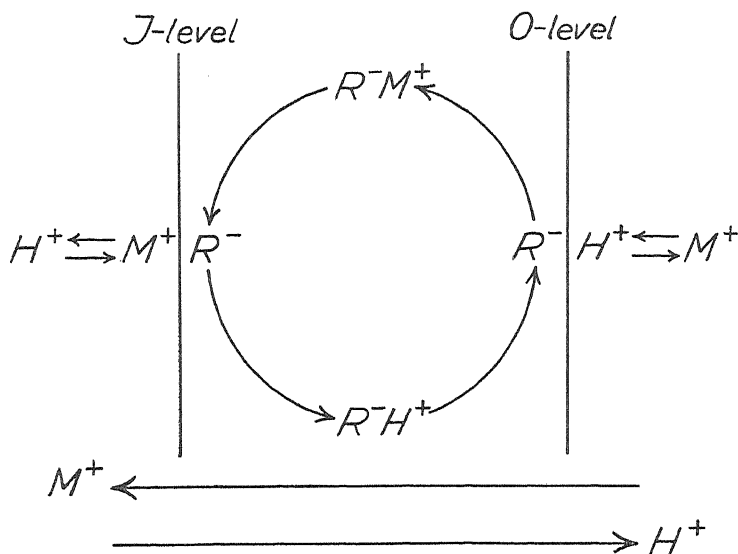


Fig. 36. Scheme of the adsorption and transport of cations. It is postulated that eH is higher at the I-level than at the O-level. This is always the fact if the anion respiration endures, but can occasionally be effected by internal production of acids (see p. 305). R^- is the hypothetical «carrier» of the cations (see the text).

established everywhere, if such a gradient exists. But a polarity can of course be thought to arise otherwise too. Our scheme is only proposed as the starting-point for fresh investigations.

This electro-chemical system is thought to work by means of displacements in the equilibrium at the levels O and I. The balance at the O-level can be expressed by the equation

$$\frac{[H^+]_{zo}}{[H^+]_o} = \frac{[A^-]_o}{[A^-]_{zo}} = k_0$$

Here zo indicates the ion concentration of the protoplasm at the O-level, and o indicates the bulk phase. If $[H^+]_{zo}$ decreases — which according to the theory is due to the anion respiration — $[A^-]_{zo}$ i. e. the absorption of anions increases.

The balance at the I-level is expressed by the formula

$$\frac{[\text{H}^+]_{zi}}{[\text{H}^+]_i} = \frac{[\text{A}^-]_i}{[\text{A}^-]_{zi}} = k_1$$

Here zi indicates the concentration of ions in the protoplasm at the I-level, and i indicates the place of accumulation. If $[\text{H}^+]_{zi}$ increases through anion respiration (see above) anions $[\text{A}^-]_i$ are accumulated. This is a very simplified assumption. The applicability of a simple membrane equilibrium is somewhat doubtful (p. 306).

The question arises why the mechanism, as suggested here, does not simultaneously transport cations in the reverse direction. One can here point to the fact that the decrease of interfacial potential at the O-level (the decrease of $[\text{H}^+]_{zo}$ in the formula) remains so limited that a real reversal from negative to positive potential does not occur. The protoplasm no doubt retains its dominating cation adsorption. If no organic cations are produced in the system, each anion transported to the I-level will therefore be balanced by one metallic cation which is liberated from the »molecular tracks». The object of the anion respiration — seen from the view-point of anion transport — seems to be the regulation of the ZcH -value to a level more suitable for the absorption of anions, without measurable screening of the cation adsorption. Through this process the absorption of mineral salts approaches the type represented by an isoelectrical adsorbent, e. g. charcoal (see FREUNDLICH, 1930).

The complicated nature of the accumulation mechanism is no doubt due to the circumstance that the positive and negative valencies, which bind the salt ions, as a first step in absorption, are separated by a number of non-charged molecules. This mosaic structure would lead to the development of »local elements», i. e. local discharges between opposite groups, if electrons (ions) could move freely through the protoplasmic membrane. This is apparently not the case: free diffusion of ions through the protoplasmic membrane exists only to a

small degree (p. 261). The development of »local elements» is consequently prevented by the immense electrical resistance in the membrane. Owing to these circumstances, ions of different charge are able to move along separate lines, »molecular tracks», which end with valencies in the surface of the membrane. This ion exchange will cause an apparent conductivity along the paths of ion movement. What I have outlined here is a kind of »structural conductivity» of the protoplasmic membrane, and this idea seems to open up a possibility of understanding the fact that anions and cations are absorbed separately and also forwarded separately through the protoplasm. This new idea is only a further development of my previous (1935 a) assumption of separate »carriers» for cations and anions. The chemical nature of these »carriers» is quite unknown, and it is even superfluous to assume specific carriers, because molecular chains of the most varied natures will be capable of transporting ions.

According to the foregoing discussion, accumulation and exudation are two sides of the same process. If the I-level in fig. 34 is conceived as the outer limit of an exudation organ, the outlined mechanism will function as an exudation mechanism. A similar system seems to work in assimilating leaves, according to ARENS (1933, 1936). In the leaves of aquatic plants potassium is absorbed at the underneath side and given off again from the upper side, according to the formula $12 \text{ KHCO}_3 = \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2 + 6 \text{ K}_2\text{CO}_3$ (see also LAUSBERG, 1935). BRAUNER (1938) found potential differences in leaves in connection with light supply, and this also supports the conception of an accumulation-exudation mechanism similar to that suggested in the scheme in fig. 34. Further research work is required here. In non-living systems also, light seems to induce potential differences (see BRAUNER l. c.).

The coupling between ion absorption and respiration does not seem to be specialized to roots. Earlier work by STEWARD, PETRIE, HOAGLAND and collaborators (see STEWARD 1935)

shows that storage tissue can also absorb salts and that this absorption is coupled with »aerobic metabolism». Even in the animal kingdom, similar phenomena have been discovered. HUF (1936) found that the uptake of Cl^- in the skin of the frog is coupled with an oxidation process. According to him, the material for this »anion respiration» is probably lactic or pyruvic acid, not glucose. A further similarity between the phenomenon described by HUF and the anion respiration of roots is the circumstance that both are checked by KCN. Potential differences have also been observed by HUF.

The ion absorption through the skin of the frog has also been studied by KROGH (1937, 1938). He claims (1937 a, p. 755) that »a mechanism closely resembling LUNDGÅRDH's is of widespread occurrence and of great biological significance in the animal kingdom». In a later paper he studied the absorption of anions and cations in frogs, goldfish and the Eriocheir crab (KROGH 1938). He finds that also one-sided absorption of cations can be promoted by special, energy-expending processes. I have previously mentioned that a special cation absorption respiration would be necessary only if the charge of the absorbing surface is decidedly electro-positive in relation to the medium. I have also pointed out that, owing to the fact that the isoelectric point of the protoplasm is usually on the acid side, the normal charge of the surface will be electro-negative, unless the medium has a very low pH or contains metallic cations in high concentration. But it might happen that special protoplasts, tissues or membranes develop a markedly electro-positive surface potential (hence negative potential of the medium-electrode, cf. p. 260). We do not know anything about that. The mechanism which has been outlined in fig. 34, has of course a transport capacity in both directions, as I pointed out above. If the adsorption capacity of the total protoplasm for cations is very low, but the capacity for anions is high (hence the reverse of the actual case in roots) this mechanism will no doubt

ject cations at the O-level. And it will be the task of future research work to show whether conditions for the work of such »reversed anion respiration mechanism» are realized anywhere. I will not omit to call attention to the fact that one-sided absorption of cations can be very simply explained by the one-sided production of organic anions at the I-level (see p. 305 and fig. 36). If this anion-production is involved in a respiration process, a »cation respiration» can be conceived. HCO_3 -ions, too, can, to a certain extent, function as receivers of metallic cations. KROGH (1938, p. 350) agrees with my conception of the absorption as an ion exchange and of »carriers», which transport the ions to the place of accumulation.

According to the theory, roots with high ZcH_{max} -value offer a higher resistance to the absorption of anions than roots with lower ZcH_{max} -value. My experience of different plants is not yet very extensive, but I have studied the four chief cereals in respect of ZcH_{max} of the lower part of the roots. Wheat has the value c. 10^{-3} and similar values are obtained for oats and barley. Only rye shows a markedly lower value, c. $\text{ZcH}_{\text{max}} = \text{c. } 10^{-4}$, according to a large number of measurements made in this laboratory.

An experiment has now been made with the anion absorption and root respiration of rye seedlings in comparison with wheat seedlings. The method of circulation vessels (fig. 17) was used. The solution was 0.0025 n KNO_3 . After six hours the quotient $\frac{\text{total respiration (CO}_2\text{)}}{\text{anion absorption}}$ was 1.7. For wheat the same quotient was 3—4. Thus the total respiration of the rye roots was below the coefficient $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ for wheat (usually = c. 2). In a 0.001 n KCl solution the relation $\frac{\text{total respiration}}{\text{anion resorption}}$ for rye was 2.1. In this case, too, the same relation for wheat is above 3. These results evidently accord with the electro-chemical theory.

A conclusion that the rye plant is more »economically»

constructed than wheat will perhaps be premature. It ought to be remembered that only a minor part of the energy expended in the anion respiration is used up by the accumulation work (p. 319). According to BURSTRÖM, the accumulation of salts is essentially confined to the zone of young, still growing root hairs (p. 284). The duration of life of the root hairs is limited, and a continued uptake of salts is therefore coupled with continued growth. A calculation of the amount of glucose consumed during the experiments, and the growth during the same time (see LUNDEGÅRDH 1937, p. 111) shows that c. 30—60 % of the glucose must be transformed into growth material. The assumption lies ready to hand that a part of the surplus energy during the anion respiration is confined to this growth material (see also ALTEN & GOTTWICK 1939). From this point of view the rye plant is perhaps not more »economical» than wheat.

The comparative studies of the ion absorption of rye and wheat were also extended to cover the cation:anion balance; for potential measurements resulted in a lower ZcH -value for rye in salt solutions too. The experiments with salt absorption were performed both in solutions and in quartz sand.¹ They will be published later. I mention here that the quotient $\frac{NO_3^-}{K}$ attained values of 0.9—1.3, hence values similar to those for wheat. Genetically conditioned differences in the charge of the surface of the root system consequently allow of no conclusions as to the relation anions:cations. The primary adsorption of ions in the surface is apparently only one of numerous factors, which determine the total ion balance. I have previously (LUNDEGÅRDH 1939 d) called attention to the fact that the plant as a whole tries to maintain a certain general balance between inorganic anions and cations, for every deviation from a balance = c. 1 means an expenditure of energy necessary for the creation of organic ions (including HCO_3^-) to counterbalance it. Experiments with nutrient solu-

¹ Unpublished results by T. BURLACU.

tions also show that each plant usually regulates the external pH-value in a certain way (usually in its own way keeping it lower than 7) and that more of one ion is absorbed, if the amount of other ions of the same charge is reduced (Mc CALLA and WOODFORD 1938). In this general balance between anions and cations the following chief factors are involved:

A. Factors which tend to increase the relation $\frac{\text{anions}}{\text{cations}}$ (of salts) in the medium.

a. The negative charge of the root system, which causes a strong primary adsorption of metallic cations.

b. Combination of fast-moving cations with slowly-moving anions (e.g. K^+ and SO_4^{--}).

c. Production of acids in the metabolism, which promotes the absorption of cations (p. 305).

d. Carbon dioxide, produced by the respiration. Its power to regulate the external pH also depends upon the aeration of the medium, conformably with the balance $[CO_2 + H_2CO_3] = K_0P$. Here P = the partial pressure of CO_2 in the air.

e. Organic or inorganic anions exuded from the roots. This seldom happens in aerobic life (anaerobically acids are given off from the roots), but is observed in corn in the dark (LUTTKUS and BÖTTICHER 1939, SCHMIDT 1936; see also ACHROMEIKO 1936) and in solutions of single salts with one-sided absorption of anions (see LUNDEGÅRDH, BURSTRÖM and RENNERFELT 1932). Theoretically an exudation of anions in the medium ought to happen with repressed anion-respiration, in association with a high negative charge of the root surface.

B. Factors which tend to diminish the quotient $\frac{\text{anions}}{\text{cations}}$ in the medium.

a. Low negative, or even positive, charge of the surface of the root system. ZcH_{\max} is of importance here (cf. rye and wheat) and also the eliminating influence of the anion respiration.

- b. Combination of fast-moving anions with slow-moving cations (e.g. NO_3 and Ca).
- c. Transformation of absorbed anions into cations or their destruction as ions. Examples: $\text{NO}_3^- \rightarrow \text{NH}_3$, $\text{SO}_4 \rightarrow \text{SH}$.
- d. Exudation of metallic cations.

From this enumeration one can see that the composition of the nutrient solution, and partly also its aeration, influences the ion balance. It shows also why a good nutrient solution must contain a suitable assortment of ions, notwithstanding the nutrient value of some of these. An unsuitable composition of the solution may lead to an irreparable lack of balance (examples in LUNDEGÅRDH 1932 and LUNDEGÅRDH and BURSTRÖM 1933 a).

The anion respiration is an important link in the general metabolism of the plant, but this is influenced by a large number of other factors too. The absorption of ions is physiologically coupled with the translocation of salts and glucose within the plant, and it is, therefore, not surprising that, e.g., the carbohydrate assimilation, and consequently the light factor, as also the transpiration, will influence the absorption (or exudation) of ions too. In order to study the chief function of the root, viz. the absorption, of ions and of water, it is therefore advisable to work with decapitated root systems, which have stored up enough glucose under constant growth conditions. But a comparison between the respiration of intact root systems and decapitated ones (see LUNDEGÅRDH 1937) showed that intact seedlings, which are grown under constant conditions of light, temperature and humidity give practically identical results with decapitated systems. We have found in this laboratory that one cannot work well with plants which are more than three weeks old, or which have grown up in glass-houses under uncontrolled conditions. HOAGLAND and BROYER (1936, p. 491) apparently have not apprehended these facts, when they criticize the methods employed by LUNDEGÅRDH and BURSTRÖM. The isolated root system works

fairly well as long as sufficient glucose, amino-acids and vitamin B₁ are present (see also BONNER and ADDICOTT 1937 and BONNER 1938).

As stated above, the study of the interfacial potentials of roots promoted a new conception of the chemical and physical nature of the protoplasmic membrane. Dissociable substances, such as phosphatides and fatty acids, cannot be assumed to be the chief constituents of the membrane. The hypothesis advanced by COLLANDER (1937) that the surface film is a film of lipoids, free of protein, cannot be accepted, if by lipoids phosphatides are meant. Owing to the high dissociation power of phosphatides, these would only be present to an amount of c. 1 % of the total number of molecules in the surface (p. 272). The main substance of the membrane is no doubt non-dissociable, e.g. fats or other substances of ester character (cf. p. 272 et seq.). The orientation of the molecules in strains perpendicular to the surface, causes a considerable resistance to water and dissolved molecules.

Our results remind of OSTERHOOT's conception of a »non-aqueous layer» (see p. 236). The large cells of algae, for which OSTERHOOT and his school show a preference, remind us in several respects of the roots. Accumulation of salts, including a marked in-equivalency of anions and cations of single salts, occurs in both. The normal surface potential (50—60 mv) occurs in both. On the other hand, OSTERHOOT and his collaborators believe that salts are »dissolved» in the protoplasm and that the partition coefficient water : protoplasm determines the absorption (see also WILBRANDT 1938).

In the roots the absorption of salts is an active process and pure diffusion of salts through the surface seems to be of little importance, whereas this possibility cannot be completely excluded. The revealing of the absorption mechanism seems to have proceeded farther in respect of roots than in respect of large cells of algae. A chief objection to the latter as objects of research is the comparatively slow metabolism and the difficulty in following quantitatively the uptake and

exudation of ions, as well as the respiration processes. The studies by WARBURG and NEGELEIN (1920), on the other hand, show that it is possible to work quantitatively with algae cultivated under controlled conditions. Some of the results of these investigators reveal surprising parallelisms to the behaviour of roots of higher plants, viz. the lack of effect on the fundamental respiration of KCN, and the sensitivity of the absorption of nitrate to this poison (see LUNDEGÅRDH and BURSTRÖM 1935, p. 242 et seq.). It therefore seems more probable that the mechanism of ion absorption and accumulation is the same in the lower and higher plants, and perhaps also in the animal kingdom (cf. KROGH).

I have just mentioned that the chemical nature of the main substance of the protoplasmic membrane is little known. If phosphatides or lipoids of similar character are present, they are certainly not the main substance. Nor can proteins be assumed to be main constituents of the surface of roots, because their dissociation would give quite different potentials from those observed (see p. 272). In respect of the physical structure of the membrane, the conception of oriented molecules is supported both by physical studies on monolayers and by our investigations on the stability of the membrane (p. 267). The permeability of dissolved molecules is quite conformable with the idea that penetrating molecules must squeeze between the long tails of the membrane-molecules. Model studies on monolayers (RIDEAL and others, see p. 286) show that chemical interaction with these tails largely promotes the penetration. From this point of view »lipoid permeability» seems quite acceptable.

The old idea of actual »pores» in the protoplasmic membrane has always been confronted with the difficulty of accepting an assortment of pores of very varying widths. Experience teaches us that there is seldom an absolute upper limit for the size of penetrating molecules. Differences in permeability are chiefly differences in the velocity of penetration. The existence of pores or holes in the membrane is *a priori* very improbable,

because the membrane is certainly not a solid, but a viscous liquid. A film of oriented molecules would function as a strainer with variable-sized holes. The resistance to penetration would be caused by the cohesion, viz. the attraction between the lipophilous tails of the oriented molecules. Large molecules would have a greater resistance to overcome in squeezing between the tails, than small ones, and this would explain the different permeability coefficients and also the advantages enjoyed by lipophilous substances. It is a fact that also very large molecules, e. g. virus molecules, penetrate the protoplasmic membrane, and that would be impossible, unless the membrane had a considerable elasticity. The nature of the force which drives such large molecules through an elastic membrane is still unknown.

According to these conceptions as to the properties of the protoplasmic membrane, the penetration of non-dissociated molecules through the membrane is to be apprehended as an interaction with the molecules of the non-dissociated main substance of the membrane. In this case all the circumstances that influence the interaction, viz. »surface activity», »solubility in lipoids», magnitude, shape (dipols) and configuration of penetrating molecules, must also affect the degree or velocity of permeability. Even small changes in the chemical composition of the main substance, which is probably a mixture of compounds, can affect this molecular interaction. It is therefore not surprising that so many different types of permeability have been found. By way of examples, I mention here SCHÖNFELDER's (1930) and HOFFE's (1933) rather different »pores» in *Beggiatoa* and *Psalliota*, in comparison to *Rhodo discolor*, and RESÜHR's (1935) conclusion that, for eggs of *Fucus*, the »lipoid theory» is more appropriate than the »strainer theory» (see also reviews of COLLANDER, WILBRANDT and others).

According to our new conception of the protoplasmic membrane, the penetration of ions would not occur through its main substance. I have based this conclusion primarily on my own experimental experience, but a number of earlier

well-known facts also support it, e. g. how slowly the small ions generally penetrate the cells in comparison to molecules. The possibility can not of course be excluded that, in special cases, ions of salts also penetrate the main substance by »diffusion», and that in such cases the principles of »partition coefficients» (OSTERHOUT) etc. might be applicable. Here I have in mind chiefly experiments with abnormally high salt concentrations, which are used with plasmolytic methods. In this connection decisive importance would attach to a statement of diffusion potentials (p. 246). If diffusion potentials are not developed, as in the wheat roots, we must conclude that the ions must enter the protoplasmic membrane by means of exchange at the distinct points in the membrane which are carriers of free valencies. The »ion permeability» will consequently be of quite a different type from the »molecule permeability», and as a rule imply transformation of energy (»metabolic processes»).

The distinction between two fundamental types of permeability does not necessitate the conclusion that intermediate cases do not occur. Here I have in mind large organic ions, e. g. of dyes, which have a charged »head» and a considerable un-charged »tail». The head will tend to be attracted to the charged points in the membrane, and some observations by COLLANDER and HOLMSTRÖM (1937) on the absorption of sulphonic acid dyes seem to show that such large ions behave similarly to small ions, viz. need the cooperation of »active» metabolic processes. But the large tail will probably also be affected by the tails of the surrounding uncharged molecules of the membrane, and as a consequence molecular forces will also intervene in the process of penetration. On the other hand, if the anion respiration changes the number of free valencies in the membrane, this will possibly also to some extent affect the hydration etc. of the surrounding main substance. The field is here open to further research work.

Changes in the charge of the membrane can also be brought about by changes in the bulk phase (for roots and single cells

= the nutrient solution). I have mentioned examples of, e. g. the varying stability and charge in single solutions of potassium, in solutions of calcium, in solutions with or without manganese etc. (p. 292 et seq.). These influences of the bulk phase on the membrane are partly of an indirect nature, e. g. the anion respiration, partly of a direct nature, e. g. the rapid interchange of cations and the influence of adsorbed metallic cations on the hydration, stability and viscosity of the membrane. To some extent the latter influences probably extend also to the non-dissociated main substance of the membrane. Such phenomena have frequently been described in the literature, and I mention a few of them here.

DE HAAN (1935) found that a number of salts (NaNO_3 , $\text{Ca}(\text{NO}_3)_2$, $\text{Co}(\text{NH}_3)_6\text{Cl}_3$) reduce the permeability to water at low concentrations, but increase it at high concentrations. In one of my earlier investigations on permeability I have shown a number of such interactions of salts in the permeability to water (LUNDEGÅRDH, 1911). DE HAAN believes in a parallelism to the behaviour of »auto-complex-systems» of phosphatides which BUNGENBERG DE JONG claims for the protoplasmic surface. HLUCHOVSKY and SEKLA (1933) also speak of an equilibrium between calcium and lecithin in the »Hautschicht» and support this view by experiments made by POLLACK (1928), who found such anions to be most poisonous to amoebae which precipitate calcium. Concerning the improbability of large amounts of lecithin in the protoplasmic membrane, see above. The »astringent» influence of calcium, on the other hand, is frequently indicated from many quarters (see also SAKAMURA and KANAMORI 1935) and is no doubt true. The same is the case with the swelling action of potassium.

Bivalent ions, such as Ca^{++} and Mn^{++} , generally attract water molecules from the surroundings and consequently dehydrate the membrane. The dehydration brings the molecules closer together and may, for this reason too, be the cause of the raised charge, which is a function of the number of valencies per unit of area. A denser structure also raises the sur-

face tension and the stability of the membrane, so that, e. g., higher charges will be tolerated (p. 250, 296). The reversed action of potassium is evidently caused by its weak attraction for the hydration water. A membrane in which the free valencies are chiefly saturated by potassium ions has a looser constitution: the electrical charge is lower and the membrane probably does not resist high charges.

The above-mentioned influence of metallic ions¹ on the physical properties of the protoplasmic membrane will presumably also affect the molecular permeability. DELLINGHAUSEN (1933) investigated the relation between the swelling effect of electrolytes and permeability of gelatine, cellophane and other artificial membranes. SCHMIDT (1936) found that the retarding influence of cations on the uptake of fructose increases in the series $K < Na < Li < Sr < Ba < Ca$. This is not a regular HORMEISTER series, a circumstance which points to secondary effects of the ions. The remarkable antagonism between K and Ca is again obvious. According to SCHMIDT different species behave differently in respect of permeability changes.

Besides of the reaction on the density of the surface layer the ions seem to have certain effects on the chemical constitution of this layer. In this connection the observation that boric acid increases the negative charge (p. 298) is interesting. Also the remarkable effect of very small concentrations of manganese salts (LUNDEGÅRDH 1939 c) on the charge ought to be noticed. It is perhaps worth mentioning that both Mn and B have catalytical effects in the cells. Boron enters as an anion. Of other anions the acetate anion also seems to have some chemical effect, because it seriously disturbs the balance anions:cations (see table 9). SAKAMURA (1934) also found that one-sided absorption of acetate had an injurious effect. For this reason, Ca-acetate is more injurious than K-acetate, since Ca is so slowly absorbed. One-sided absorption of anions from diluted mineral acids also seems to have detrimental, and specific, effects on roots (see p. 297 and fig. 12).

¹ As is well known, large ions have weak hydration power.

It must always be difficult to decide whether an observed change in the properties of the protoplasmic membrane is due to a change in the molecular structure of the membrane itself or to interrelations with the underlying layers of the protoplasm. The membrane is certainly rather sensitive to gravitation (geoelectrical response, see p. 276). It is, furthermore, sensitive to light (see BRAUNER 1936, PYRKOSCH 1936, MEISCHKE 1936, ORTH 1937). Certain wave-lengths affect the permeability to water, sugar etc. It is quite possible that these effects are due to molecular changes within the membrane itself, e. g. the specific weight of ions (with geoelectrical response, see BRAUNER) or ionization by radiation. Further non-living systems of colloids plus salts show changes in the »permeability», which are induced by gravitation or light (see also BRAUNER 1936, BROOKS 1923). I would call attention here to the observations made by SVEDBERG and BROHULT (1939) on the splitting of large protein molecules by illumination with ultraviolet light. From a physico-chemical point of view the perception of light can be referred to such effects on the molecular structure or charge, and it is therefore not surprising that changes in the potential of perception organs are observed (see AMLONG and BÜNNING 1934, BÜNNING 1939).

The question whether the metallic cations only have a »physical» effect on the permeability, the potentials etc., according to their size, charge and hydration power, or more profound »chemical» effects, is frequently discussed (see LUNDEGÅRDH 1932). If physical effects prevail, one cation might be able to replace another cation of similar physical properties. In several respects Rb and Cs can replace K, while Li can function as a substitute for Na (PIRSON 1939). On the other hand, potassium seems to have specific chemical effects too, e. g. on carbohydrate assimilation (PIRSON 1937). This more »chemical» effect of cations is in many cases probably due to a catalytical power. The action of Mn as a catalyst has been studied by BURSTRÖM (1939, nitrate reduction) and LUNDE-

GÅRDH (1939 c; fundamental respiration of roots). Earlier experiments by LUNDEGÅRDH and BURSTRÖM (1935) showed specific »chemical» effects for a number of heavy metals, e. g. Tl, Hg, Pb etc. on respiration and ion absorption (earlier researches on the poisonous effects of heavy metals, see BURSTRÖM 1929, FIRSCHLE 1934, 1935 a. o.).

BURSTRÖM (1939) found that the catalytically active manganese is bound interchangeably, viz. »adsorptively», so that the catalytical action disappears, if Mn is replaced by Fe or Ca. This interesting statement recalls observations on enzymes as composed by a carrier and a prosthetic group and calls forth the question, whether a single heavy atom can act as a prosthetic group. Chemically, as is well known, it is possible to substitute atoms, e. g. the Fe atom in chlorophyll for other heavy atoms (see HANSON 1939); the chemical characteristics are then also changed. If catalytically active, heavy metals can develop their qualities in interchangeable binding, this would perhaps render possible an explanation of the fact that a large number of »dispensable» ions, e. g. Zn, Mo etc. show stimulation effects in certain dilutions. These heavy ions would then perhaps enter as prosthetic groups in linkage with specific proteins, and the total effect of the »enzyme» would then depend upon the influence of the metallic atom. The assumption that these general effects of heavy metals on growth etc. are due to atoms (or ions) in interchangeable binding is supported by the fact that antagonistic effects are also observed between heavy metals (GOLLMICK 1936).

The effect of very small amounts of Mn^{++} on the surface potential of roots has already been mentioned (p. 296). In this case the potential was increased, whereas a simple substitution of H-ions against Mn-ions would lower the potential. I called attention to the fact that the »astringent» effect of ions of high hydration power is possibly a contributory cause of that. But the rise of the potential might also be caused by secondary chemical processes, induced by the heavy ions. As

a matter of fact, another heavy ion, Cu^{++} , has a reversed effect in comparison with Mn^{++} . In an experiment the roots were treated with a 10^{-5} n solution of CuCl_2 . After 30 minutes the measured potential was found to be c. 20 mv *lower* than the controls. Cu must, then, act in a quite different manner from Mn. Earlier experiments (LUNDEGÅRDH and BURSTRÖM 1935, p. 236) showed that Cu has a specifically stimulating effect on the fundamental respiration! These observations show that the problem of heavy metals is complicated and needs continued investigation.

Summary.¹

Experiments were carried out for the purpose of elucidating the interaction of aerobic respiration of roots and the electro-chemical behaviour of the surface of the protoplasm. The results are discussed on the basis of our knowledge of the physical and chemical properties of the protoplasm, especially its surface layers, and support an electro-chemical theory of the absorption and accumulation of ions in living cells and organs.

1. Absorption, accumulation and exudation are apprehended as different aspects of the same mechanism. This mechanism involves the ability of the living body to transport ions faster than pure diffusion would do. The mechanism has aerobical energy-expending processes at its disposal and is, accordingly, able to overcome very high osmotic resistances. A possible relation between the absorption work and the hydrostatical pressure of the bleeding sap is discussed.

2. In the normal life of roots of the plants investigated the absorption of ions is coupled with an aerobic respiration process, the »anion respiration», which involves a complete combustion of glucose $\left(\frac{\text{CO}_2}{\text{O}_2} = 1\right)$. This process is rather sen-

¹ Cf. the definitions on p. 234.

sitive to the oxygen supply. A small ion absorption is also observed in anaerobic life, but is anticipated to be of little importance for the normal life of the roots. The anion respiration is sensitive to KCN. Simultaneously KCN also checks the absorption of anions.

3. A semi-quantitative relation exists between the anion respiration and the absorption of anions. Under otherwise constant conditions, the coefficient $k = \frac{\text{anion respiration}}{\text{anion absorption}}$ is fairly constant, if the intensity of the process is varied by limited variations in the concentration of a single salt in the solution. k is an expression for the osmotic (electro-chemical) work which is needed for the accumulation of one unit of anions from outside. As a consequence of that, k increases with decreasing mobility of the anions in the organ, with increasing concentration gradient and with increasing negative electrical charge of the surface of the root system.

4. The fact that the energy-expending process (anion respiration) is coupled exclusively with the absorption of anions is proved by a large number of experiments, in which anions of different mobility have been combined with cations of different mobility. Electro-chemically the accumulation of a salt (anion + cation) is exhaustively explained by the active accumulation of only *one* of the ions.

5. When the anion respiration supports an active absorption of the anions of a salt, its cations are passively attracted. This is the reason why, on an average, the absorption of cations runs parallel to the absorption of anions. But in absorption during 1—20 hours from a single salt the quotient $\frac{\text{absorbed anions}}{\text{absorbed cations}}$ is only exceptionally = 1 and, by combination of ions of different mobility, can be varied within very large limits. Nevertheless the anion respiration usually runs parallel to the quantity of absorbed anions. The fact that when salts with similar mobility of anions and cations are used, the anion respiration also runs parallel with the ab-

sorption of the cations is evidently not in contradiction to the theory of anion respiration.

6. If, according to 3., the coefficient k is an expression for the work of transporting anions against an osmotic gradient, the mobility of the cations in the transporting track must also participate. One-sided absorption of anions gives rise to large potential differences between the O-level and the I-level. Cations of high mobility will, for this reason, lower the expenditure of energy, hence the coefficient k , cations of slow mobility will raise k . According to this electro-chemical postulate, the cations of a salt increase the coefficient k in the order $\text{Na} < \text{K} < \text{Mg} < \text{Ca} < \text{Sr} < \text{Ba}$.

7. The *fundamental respiration* is the totality of combustion processes which are not directly¹ involved in the absorption of ions. The fundamental respiration is characterized by its insensitivity to KCN and the fact that manganese is one of the catalytical factors. The fundamental respiration is furthermore characterized by its ability to utilize very small partial pressures of oxygen.

8. The amount of glucose, stored in seedlings exposed to strong electric light during 18 hours a day, is usually sufficient for maximal anion respiration during at least 6 hours. A lowering of the available glucose also affects the anion respiration. Feeding with glucose from outside affects primarily the fundamental respiration. Only exceptionally is the anion respiration increased by the feeding of normally cultivated seedlings. Feeding with glucose promotes the production of acids. As a consequence feeding with glucose can promote an one-sided absorption of cations.

9. The intensity of the respiration and the absorption of anions are different at different heights of the roots of wheat. The ends of the roots (0—30 mm) have a much higher respiration per unit of dry weight than the upper parts. Also the absorption of anions is highest in the ends, but the difference is not so large as in respect of the total respiration.

¹ Reservation is made for the case when acids are produced (see p. 305).

10. The surface of the root system of cereals is normally charged electronegatively in comparison to the nutrient solution. The charge amounts to c. 50—60 mv. In a very diluted solution of mineral acids the charge amounts to 150—200 mv. This charge is a property of the protoplasmic surface of the epidermis cells and is not due to the cellulose wall.

11. Carriers of the charge are negative valencies in the surface of the protoplasm. In the absence of metallic cations in the bulk phase these valencies are saturated only by H-ions and the density of the valencies, expressed as ZcH_{\max} , can be approximately calculated from measurements of the interfacial potential in pure, diluted acids.

12. If metallic cations are present in the bulk phase, a partial exchange occurs with the H-ions of the surface layer of the protoplasm. This exchange follows simple laws of adsorption. As a consequence the interfacial potential decreases.

13. The interchange of cations at the surface layer follows known laws of hydration power, charge etc., of the metallic cations.

14. Potential measurements favour the assumption that anions of neutral salts are adsorbed to the surface layer of the protoplasm by exchange. Hence the surface layer also possesses positive valencies, which in the absence of anions of acids are saturated only by OH- (and a small amount of HCO_3^-) ions.

15. The protoplasmic surface of a wheat root in normal nutrient solutions is occupied by large amounts of metallic cations (being an assortment of all the salt cations), a much smaller amount of H-ions (symbolizing the charge), and minute amounts of OH-ions and anions of neutral salts.

16. The interchange of cations and anions at the surface of the protoplasm is assumed to be the first stage of absorption.

17. The oligodynamical affect of heavy metals in distilled water is due to the very high adsorption potential in the absence of other metallic cations.

18. The surface layer of the protoplasm is conceived as being built up of oriented molecules, in analogy with one or a few monolayers. The main substance of the layer consists of non-dissociated molecules, and the molecules which carry valencies lie like islands among these other molecules. Positive and negative valencies are probably not carried by the same molecules, but form a sparse mosaic. The molecules which carry the negative valencies have a high dissociation constant, and the hypothesis is advanced that they are phosphoric acid groups, attached to some organic compound.

19. The stability of the surface layer depends, among other factors, upon the interfacial charge: A discharge of the layer deranges the stability and so does also a too high charge. Adsorbed cations affect the stability in a similar way, which is well-known from colloids. Potassium lowers the stability, calcium raises it.

20. A causal relation exists between the height of the electro-negative charge of the root, the intensity of the respiration and the absorption of ions. An electro-chemical theory of the absorption and accumulation of ions in living cells and organs is developed.

21. A high surface potential of the roots is promoted by factors which check the respiration. All factors which promote oxidation (oxygen, oxidative agents, feeding with glucose) lower the negative potential.

22. A high surface potential, which under aerobic conditions is maintained by interchange of ions, induces a high intensity of respiration. Roots of high ZcH_{\max} (wheat) show higher k -values than roots of lower ZcH_{\max} (rye).

23. An artificial rise of the surface potential due to the application of added currents induces an increased respiration, accompanied by a decreased absorption of anions.

24. The differences in the respiration which are induced by differences in surface potential, make it probable that only the anion respiration is affected, not the fundamental respiration.

25. The linkage between anion respiration and potential removes the obstacle which the negative charge of the surface of the root raises against the absorption of anions.

26. The anion respiration of roots is apprehended as a redox system, which is balanced by (a) the surface potential and (b) the transport of anions from the O-level to the I-level. (See also points 3—6). The formula $R_t = R_g + k \cdot A$ represents an ideal limit for the linkage between the respiration and absorption of anions, which is valid only when the surface potential is approximately constant.

27. Permeability problems are discussed in the light of the new conception of the protoplasmic membrane.

28. The charge of the surface of a single root is not uniform. A maximum of negative charge occurs in the zone of elongation. Between this zone and the tip differences of some 10 mv are observed. The possibility of the presence of local currents within the apical part of the growing root and their power to transport anions (e. g. auxin) is discussed.

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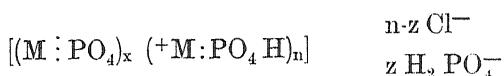
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Cationic complex with z saloid-bound H_2PO_4^- ions.

These saloid-bound H_2PO_4^- ions account for the GAARDER effect, i. e., a maximum in the phosphate fixation on the acid side of the I. E. P.

The saloid-bound ions must be easily displaceable especially by the divalent SO_4 ions, that is, the GAARDER effect must disappear in the presence of a sufficient concentration of SO_4 ions. GAARDERS own results give us a hint of such a displacement, for although GAARDER did not make special additions of salt to his series he did, in certain cases, change from the use of AlCl_3 to $\text{Al}_2(\text{SO}_4)_3$ as a reagent.

Fig. 44 shows the PO_4 fixation in three of GAARDERS aluminum phosphate series. Curve I shows the fixation when Na_2HPO_4 reacted with AlCl_3 and curve II when $\text{Al}_2(\text{SO}_4)_3$ was substituted for AlCl_3 . We note that the GAARDER effect (the depression in the phosphate solubility on the acid side of the I. E. P.) is appreciably smaller in the sulphate (II) than in the chloride (I) series. Curve III shows the fixation in a series in which the sulphate was used but in which the NaOH used to adjust the pH was added to the aluminum solution before the addition of the phosphate. The colloid-binding was therefore here less complete thus leaving a greater concentration of phosphate ions for the saloid-binding. The GAARDER effect is therefore greatest in this series in spite of the presence of SO_4 ions.

In his work on the isoelectric precipitates MATTSON (1930) found an increase in the PO_4 fixation in a ferric phosphate system not only on the acid (positive) side of the I. E. P., as did GAARDER, but also on the alkaline (negative) side as well. This was never satisfactorily explained and it became one of the many problems which, with good intentions, are put aside to be »studied later». It should be mentioned in this connection that MATTSON made his positive and negative sols 0.2 N

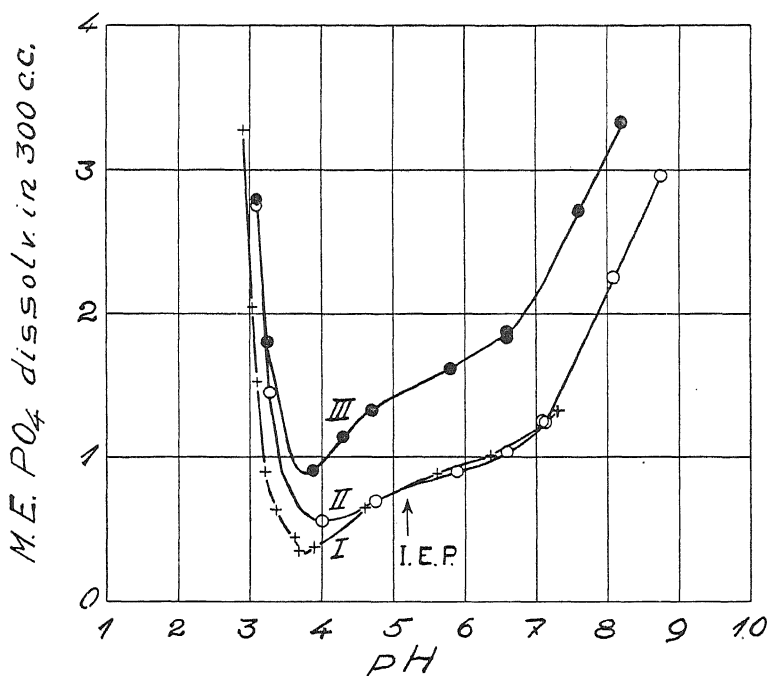


Fig. 44. The solubility of PO_4 in systems of equivalent quantities of PO_4 and Al when Na_2HPO_4 solution was added:

- I. to AlCl_3 solution;
- II. to $\text{Al}_2(\text{SO}_4)_3$ solution;
- III. to $\text{Al}_2(\text{SO}_4)_3 + \text{NaOH}$ (sol).

From GAARDER.

with respect to NH_4Cl in order to cause flocculation. This incidental fact explains, as we shall see later, why the fixation was found to be greater on both the positive and the negative side than at the I. E. P. where no salt was added.

Experimental.

In order to find out how the presence of salts affect the two forms of binding of the phosphate ion the precipitation was carried out in water (I) and in 0.5 N solutions of Na_2SO_4

(II), NaCl (III) and KCl (IV) under otherwise identical conditions (cf. fig. 45). The precipitation was done by rapidly mixing a series of solutions *A* and *B* of the following composition:

<i>A</i> :	—	7.5 m. e. AlCl_3	
		H_2O	to make 250 cc.
<i>B</i> :	—	8 m. e. Na_2HPO_4	
		250	» salt (in series II, III and IV)
		x	» NaOH
		H_2O	to make 250 cc.
			<hr/>
			= 500 cc.

After 4 days during which the mixtures were repeatedly shaken they were filtered through a ZSIGMONDY ultra filter (pores = 100–200 m μ). The filtrates were then analysed for PO_4 by the method of ZINZADZE (1935) and for Al by the ortho-oxy-quinoline method. The results are plotted in fig. 45.

In these series we used somewhat more than one equivalent of PO_4 to one of Al for the purpose of increasing the saloid binding. The concentration is otherwise of the same order of magnitude as that in our previous study and as that of GAARDER who used about 5 milliequivalents in 300 cc. For the sake of comparison we are expressing the results in terms of milliequivalents in solution in 300 cc.

The curves in fig. 45 bring out the following significant facts:

1. Where no salt is added there is a considerable GAARDER effect (a maximum in fixation on the acid side of the I. E. P.).

2. The GAARDER effect vanishes in the presence of the sulphate and is greatly reduced by the chlorides.

3. On the acid side the solvation and solution of Al and PO_4 takes place simultaneously and at a somewhat higher pH (up to 0.25 unit) in the chloride solution than in the absence of added salt and at a much higher pH (up to about one unit) in the presence than in the absence of sulphate.

4. At the I. E. P. (which lies at about pH 5 in the salt-

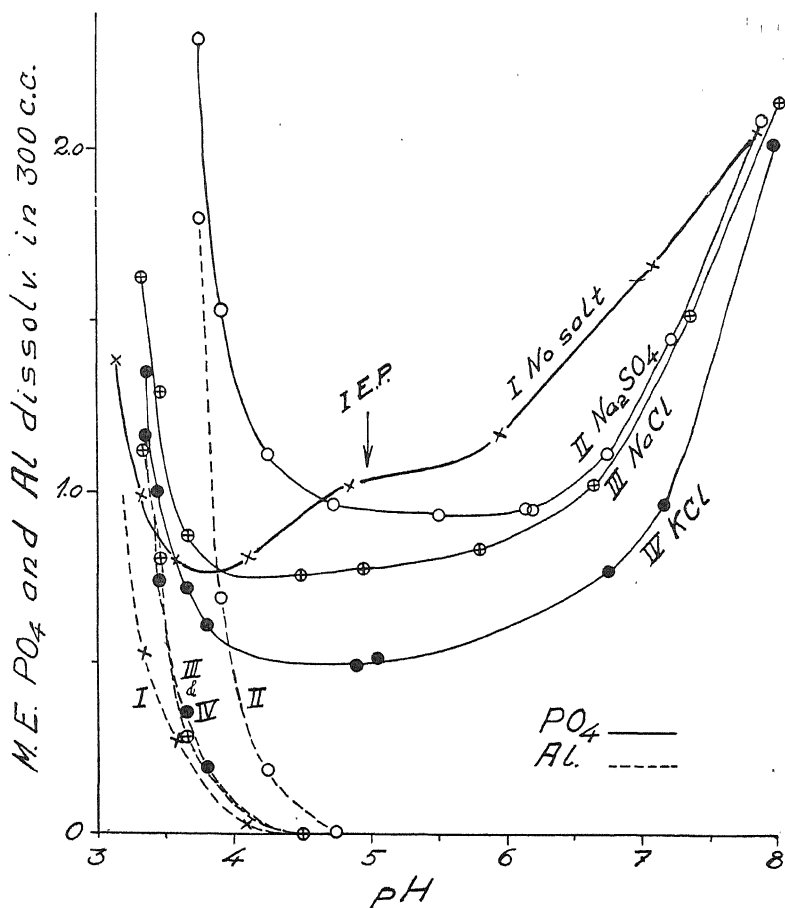


Fig. 45. The effect of salts on the solubility of PO_4 in systems of phosphated alumina containing a small excess of PO_4 .

free series) all of the salts cause a decrease in the PO_4 solubility, the effect being greatest in the KCl series and smallest in the Na_2SO_4 series.

5. On the alkaline side of the I.E.P. and up to about pH 8 (where alumina is isoelectric) the salts continue to depress the solubility of PO_4 in the same order as at the I.E.P. The depression amounts to about 50 % in the KCl solution

and to about half as much in the NaCl solution over a wide range on the alkaline side of the I. E. P.

6. There is no Al in solution on the alkaline side of the I. E. P. below a pH of 8. (In the series to which no salt was added there was a partial solvation at pH 7.08 and 7.85 but no particles passed the ultra-filter.)

The effect of salts on the phosphate complex is apparently very complicated and resolves itself into two distinct phases:

1. On the acid side at some distance below the I. E. P. the salts cause a more rapid dissolution of the complex. The nature of the cations (Na or K) is without effect, the reaction being dominated by the valence of the anions; the divalent SO_4 ions producing, by far, the greatest effect.

2. Beginning at some distance below the I. E. P. and extending far up on the alkaline side of this point the salts give rise to a considerable fixation of the phosphate ions. This effect is dominated by the nature of the cations, the effect of the K ions being about twice as great as that of the Na ions whereas the nature, including the valence, of the anions plays a lesser roll (the considerable difference in the effect between Na_2SO_4 and NaCl at and near the I. E. P. may be ascribed to a lag coming from the still greater difference on the acid side).

The effect of the salts on the acid side of the I. E. P. is not difficult to explain. At low pH there is a suppression of the dissociation of the PO_4^{--} and HPO_4^{--} ions. The H_2PO_4^- dominate and the complex can bind a greater number of phosphate ions per mol alumina. This gives rise to the GAARDER effect as long as the alumina remains bound to a sufficient number of di- and trivalent phosphate ions. These ions do not dissociate appreciably and do, therefore, not impart a positive charge to the complex. But at still lower pH more and more of the phosphate ions change into the saloid-bound (dissociated) monovalent H_2PO_4^- ions, the charge increases and the complex solvates and, finally, dissolves in the form of single ions. In the presence of salt the phosphate

ions are partially displaced by the anions of the salt. The divalent SO_4 ions have a greater effect than the Cl ions. Since these ions all enter into the saloid-bound union with alumina the solvation and solution will occur at a higher pH. The cations have no effect.

The effect of salts on the phosphate complex at and above the I. E. P. might seem contrary to the modern concepts of solution chemistry. Thus the solubility of a precipitate is usually greater in a salt solution than in pure water. The presence of the salt lowers the activity coefficient of the ions of the precipitate and since the product of the activity of these ions should be a constant in a solution in equilibrium with the solid phase, the solubility must increase.

In the case of our phosphate system the added salts must produce the same effect and render the precipitate more soluble. But our precipitate is not of the classical type of precipitate possessing a definite composition and structure, at least not at the active interface. It is not a stoichiometric aluminum phosphate but is better described as a phosphated alumina, a complex possessing acidic and basic residues and interacting amphotERICALLY with every ion in solution. It is in this fact that we have to look for an explanation to the observed salt effect.

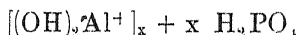
The liberation of the phosphate ions in these compounds is primarily the result of a hydrolysis and not of a solution. On the acid side of the I. E. P. the hydrolysed, undissociated acid is soluble and passes into solution with the ionized base whereas on the alkaline side the hydrolysed base is insoluble leaving the ionized acid alone to dissolve. On the alkaline side the reaction amounts to an isoelectric decomposition because the loss of the ionized acidoid group causes the I. E. P. to be deflected toward the higher pH of the system thus leading to a more stable complex. If both acid and base were colloidal in the free state (i. e., Al-humate) the complex would decompose isoelectrically on both sides of the I. E. P.

The strength of weak acidoids and basoids a function of the Donnan equilibrium.

If the liberation and fixation of phosphoric acid by the phoshate complex is thus the result of a hydrolytic reaction then the observed effect of the salts must be an effect on this reaction. A suppression of the phosphate solubility must mean a suppression of the hydrolysis of the complex by the salt and this again must mean that the ions of the salt have a power to strengthen the acidoids and basoids of the complex. In previous work (MATTSON and WIKLANDER 1939) on the pH and the capacity of soils to bind acids and bases in various salt solutions this latter fact was emphasized and an attempt was made to place the relationship on a quantitative basis in the form of the Donnan equilibrium.

The fact that the strength of acidoids and basoids is never constant but a function of the ionic environment as expressed by the activity and valence of the ions leads to many important consequences among which the fixation of phosphate is one. The connection between the two might best be explained as follows:

The acidoid and the basoid groups (e. g.: PO_4H and OHAl:) of the phosphate complex dissociate in pure water only a few H and OH ions respectively (cf. equation A below). The compound $[\text{Al:PO}_4]_x$ is therefore relatively easily hydrolysed to $[\text{OHAl:PO}_4\text{H}]_x$. The phosphate group in the latter is more soluble than in the former because the next step in the hydrolysis leads to



in which the phosphate ion is saloid-bound and subject to displacement by any other anion.

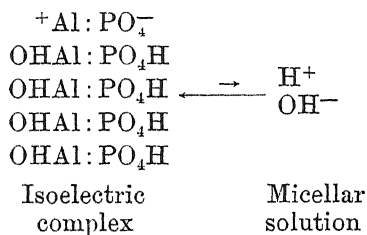
In the presence of a salt, e. g., KCl (cf. equation B below) the dissociated H and OH ions (assumed to be present in the form of an »amphoteric» ion atmosphere, the H ions, as »clouds», over the acidoid groups and the OH^- ions over the

basoid groups) are displaced by the ions of the salt in accordance to the Donnan equilibrium. This disturbs the equilibrium between the dissociated and undissociated H and OH ions resulting in an extensive dissociation of the acidoid and basoid groups. The displacing K and Cl ions are all saloid-bound and leave the colloid dissociated. At the equi-ionic point (where the K and Cl ions displace an equal number of H and OH ions respectively) the exchange can proceed very far because then a number of H and OH ions are withdrawn from the competition by the formation of H_2O : the exchange acidity is neutralized by the exchange alkalinity.

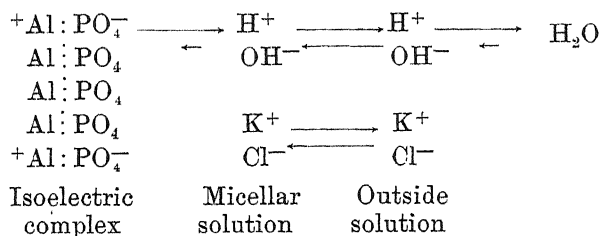
This process leaves the acidoid and the basoid groups more dissociated, that is, the acidoids and basoids have become stronger through the action of the salt. The result of this will be that, in the case of our phosphated alumina, a greater number of Al and PO_4 will unite in a triple linkage and thus fix the PO_4 more firmly.

The following two equations might serve to illustrate the relationship:

A. — In pure water:



B. — In KCl solution:



The fact that KCl has a much stronger effect than NaCl is in line with the greater displacing power of the K ions and proves that the cations exert a dominating effect at and above the I. E. P.

On the acid side the SO_4 ions show a greater displacing power and cause a solvation and solution of the complex at a higher pH than do the Cl ions. Why then does not Na_2SO_4 cause a greater suppression of the phosphate solubility than NaCl at and above the I. E. P.? If the SO_4 ions are »completely» dissociated (saloid-bound) by the complex, as postulated, they ought to show a greater effect than the Cl ions. We shall here only refer to the work of MATTSON (1930) who found that isoelectric alumina contained much more SO_4 when precipitated from the sulphate than Cl when precipitated from the chloride and that the SO_4 ions partially displaced the SiO_3 ions in the isoelectric silicate. It might also be recalled that the SO_4 ion lowers the I. E. P. considerably. We are, therefore, forced to the conclusion that although the SO_4 ions activate the alumina they form a more closely associated compound (slightly dissociated basic sulphate) than do the Cl ions and thus displace some of the double-bonded phosphate ions.

The curves in fig. 15 express the net result of several opposing forces, an exact analysis of which would require a knowledge of the activity of all the ions, colloidal as well as single, in the micellar and in the outside solution.

At a pH about 8 the curves intersect once more. The salts increase here only the dissociation of the acidoid, that of the basoid being suppressed by the high OH ion concentration. There can, therefore, be no appreciable suppression of the solubility of the phosphate ion through an increase in linkage to the alumina. Above this pH the suppressing effect of the salts upon the activity coefficient of the ions dominates the reaction and results in the »normal» effect: an increased solubility.

The influence of the composition of the complex.

We shall now see how the salt effect is related to the nature of the sesquioxide and to the degree of phosphate saturation.

Table 22 shows the phosphate »solubility» in water and in 0.5 N KCl solution of two samples of phosphated alumina and two of phosphated ferric hydroxide of varying composition. The samples were prepared ten years ago by isoelectric precipitation (MATTSON 1930), and had been freed from electrolytes by washing and by electrodialysis. They were shaken in the machine during 8 hours at a constant room temperature of 20° C. in the proportion of 1 gram per 100 cc, the pH in three of the series (the most acid ones) being slightly increased by adding a little alkali. This adjustment of the pH was done to avoid the GAARDER effect and to keep well within the range of the Donnan effect (cf. fig. 45). The ultra filtrate was analysed as in the preceding experiments.

Table 22.

The PO_4 solubility of isoelectrically precipitated aluminium and ferric »phosphates» in water and in 0.5 N KCl (1:100).

Composition	I. E. P. ¹	NaOH m. e./g	pH in:		P_2O_5 mg./l.		$\frac{s}{w}$
			water	N/2 KCl	water w	N/2 KCl s	
$\text{Al}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5) 0.897$	4.85	0.1	5.75	3.85	37.2	20.2	0.543
$\text{Al}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5) 0.490$	6.4	0.0	6.20	5.00	0.24	0.08	0.333
$\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5) 0.971$	3.7	0.1	5.35	2.90	30.0	6.95	0.232
$\text{Fe}_2\text{O}_3 \cdot (\text{P}_2\text{O}_5) 0.494$	5.5	0.04	6.50	3.95	2.16	0.11	0.051

¹ The I. E. P. values are only approximations because these precipitates were obtained in less dilute systems than those in which the I. E. P. was determined. This caused more PO_4 to be precipitated and the I. E. P. to be lower. Furthermore, the values refer only to the original wet precipitates. The I. E. P. of the dried and electrodialysed material has not been determined.

It will be noted that the KCl reduces the PO_4 solubility to as much as 0.051 of the solubility in pure water in the case of the most basic ferric phosphate. That the effect is relatively greater the lower the proportion of PO_4 is in line with the manner of reaction of colloidal electrolytes in general (cf. fig. 3 and 6 in this series). The salt effect seems to be greater in the ferric than in the aluminum phosphate. This might be partly due to a greater stability (lower dissociation) of the former which shows a lower PO_4 solubility than the latter in spite of the fact that ferric hydroxide is the weakest base. It is probably also due to a greater acidic residue in the ferric phosphate as expressed by a greater capacity to bind base and exchange cations. Note in this connection the enormous exchange acidity which in the two ferric phosphates amounts to about 2.5 pH units. The greater the displacement of the H by the K ions the greater is the dissociation of the acidoid and the greater, therefore, the number of bonds between the latter and the basoid.

We must however avoid drawing any too definite conclusions on the basis of these differences. With only a single determination, each at a different pH, it is impossible to separate the factors and tell how much of the effect is due to a change in the pH and how much it is a Donnan effect. Thus the relatively large amount of PO_4 present in the water extract of the most basic ferric phosphate (2.16 mgm compared to only 0.24 in that of the corresponding Al-compound) leads us to suspect that we are a good way up on the »alkaline slope» of the curve and that the salt effect is here partly a pH effect. A more detailed study is here highly desirable. The quantities of the precipitates on hand allowed, at the present time, only the single set of experiments here reported.

The salt effect on the calcium phosphates.

It is interesting to compare the solubility of calcium phosphates with that of the amphoteric precipitates. The following compounds were included in this investigation:

$\text{Ca}_3(\text{PO}_4)_2$ (Merck's puriss. sicc. U. S. P. VIII),
 $\text{CaHPO}_4 \cdot 2 \text{H}_2\text{O}$ (Merck's puriss, D. A. B. 6),
 Apatite, powdered (0.2—0.074 mm).

Two grams of the phosphates and 10 grams of the apatite were shaken for 8 hours in 200 cc volumes of freshly boiled distilled water and of 0.5 N KCl solution at a temperature of 20° C. The suspensions were then ultra-filtered and analysed with the results shown in table 23.

Table 23.

The solubility of tertiary and secondary calcium phosphates and of powdered apatite in water and in 0.5 N KCl.

Substance	pH in:		P_2O_5 mg./l.		s/w
	water	N/2 KCl	water w	N/2 KCl s	
$\text{Ca}_3(\text{PO}_4)_2^1$ (1:100)	6.63	6.71	108	215	2.0
$\text{CaHPO}_4 \cdot 2 \text{ aq.}$ (1:100)	6.95	7.40	80	195	2.4
Apatite (5:100)	6.87	6.88	0.4	0.8	2.0

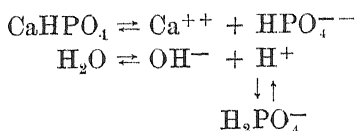
We note that, in contradistinction to the amphoteric phosphates, the phosphates of calcium are more soluble and yield a higher pH in the salt solution than in pure water. The greater solubility in the KCl solution is in agreement with the modern theory of ion activity according to which the concentration c of a solution of a slightly soluble salt in equilibrium with the solid phase must increase in proportion to the decrease of the mean activity coefficient f of the ions so that the product $c \cdot f$ remains constant, thus:

$$[\text{salt}]_1 f_1 = [\text{salt}]_2 f_2 \text{ etc.}$$

where the brackets signify concentration.

¹ For the tertiary phosphate this solubility, which was checked by a duplicate, is too high and indicates the presence of other compounds as does also the relatively low pH.

The increase in pH is obviously the result of a hydrolysis of which the following might be given as an example:



Salt effect and combining capacity.

We shall now present some experimental evidence in support of our theory that the depression of the hydrolysis of the amphoteric phosphates is due to an increase in the dissociation of the acidoids and basoids caused by the salt.

Fig. 46 shows the titration in water and in 0.5 N K_2SO_4 of the sample of Al-»phosphate» having the composition $\text{Al}_2\text{O}_3(\text{P}_2\text{O}_5)_{0.40}$ (cf. table 22).

We note that at pH 7 the complex binds about seven times more base in the 0.5 N salt solution than in pure water.

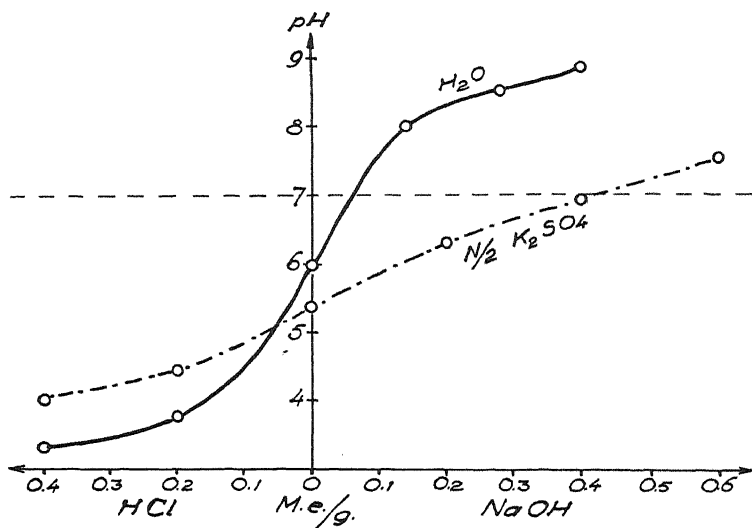


Fig. 46. The capacity of a sample of phosphated alumina [comp. = $\text{Al}_2\text{O}_3(\text{P}_2\text{O}_5)_{0.40}$] to bind acid and base in water and in 0.5 N K_2SO_4 .

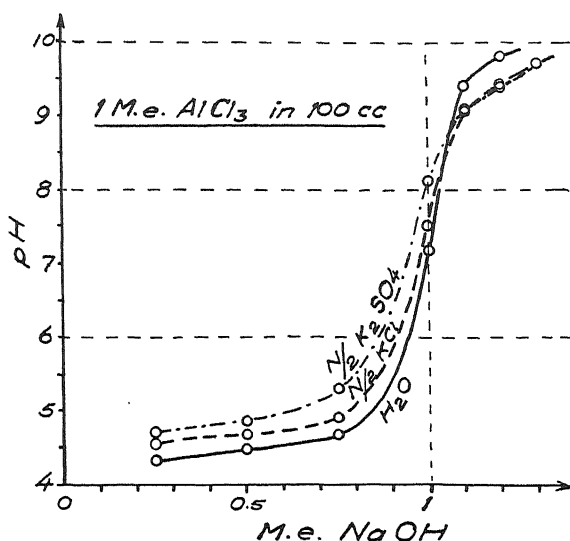


Fig. 47. The titration of $AlCl_3$ in water and in 0.5 N KCl and K_2SO_4 solution.

Assuming the acidoid to be weak and the saloid to be completely dissociated this means that the dissociation of the phosphoric acidoid is about seven times greater in the salt solution. On the acid side we note the same effect with respect to the capacity to bind acid. The two effects overlap considerably so that at the I. E. P. we can picture the complex as a highly multivalent »Zwitterion». *The depression of the hydrolysis must be greatest within that range of pH in which the product of the adsorbed anions and cations of the salt is greatest.*

The electrometric titration of $AlCl_3$ (1 m.e. in a constant volume of 100 cc) in water and in 0.5 N KCl and K_2SO_4 yields the curves in fig. 47. The curves show that on the acid side of the point of exchange neutrality (the point of intersection of the salt curve with the water curve) the $AlCl_3$ neutralizes less base in the salt solutions than in pure water at a given pH. This means that the alumina is less hydrolysed and more ionized in the salt solutions than in water. The

effect of the SO_4 ion is greater than that of the Cl ions. On the alkaline side the alumina, now appearing as an acidoid, is activated by the cations of the salts, the anions being without effect.

These phenomena are all due to a displacement of the acidoid H ions and the basoid OH ions by the cations and anions of the salt according to the Donnan equilibrium. *Therefore, weak acidoids and basoids are stronger and their compounds less hydrolysed in salt solutions than in water.*

Applications.

While this paper is being prepared an important contribution on the effect of salts on the solubility of soil phosphates has appeared in this journal (ERIKSSON 1940). After reviewing the somewhat conflicting results obtained by other investigators, from the classical work of Cameron and coworkers up until the present time the author shows, by pot experiments and by soil studies, that KCl causes a decided fixation of the phosphate in soils rich in sesquioxides. This work has established the fact that, at least in certain type of soils, the Donnan depression of the hydrolysis of amphoteric phosphates is a factor which must be recognized in ordinary fertilizer practice. That experimental results may often appear to be conflicting as long as we have not succeeded in isolating the phenomena will be clearly understood after a first attempt to apply the already established principles to soil conditions:

In our previous work we distinguished between micellar-binding, i. e., saloid and colloid-binding, which takes place on the acid side of neutrality, and extra-micellar-binding, i. e., the formation of calcium phosphates which occurs on the alkaline side. Between these minima in phosphate solubility there is a maximum in the neighborhood of the neutral point. The presence, the magnitude and the position of this maximum will depend on:

- 1) the I. E. P. of the soil complex, i. e., the acidoid/basoid activity ratio;
- 2) the amount of calcium and
- 3) the amount of phosphate present in the soil.

When a neutral salt (e. g., KCl) is added to a soil at a pH above the equi-ionic point there will be a lowering of the pH (exchange acidity).

There is at least four ways in which the salt can effect the »solubility» of phosphoric acid:

- 1) Through the depression of the pH resulting:
 - a) in a decrease in solubility on the acid side of the solubility maximum (followed by an increase at very low pH;
 - b) in an increase in solubility on the alkaline side when the depression of the pH does not extend to the acid side of the solubility maximum;
 - c) in no change when the pH of the soil is such that the effect of a) compensates the effect of b).
- 2) Through the Donnan effect leading to a depression of the hydrolysis of the amphoteric phosphates. This will occur below a pH of about 8 (the I. E. P. of alumina) down to very low values.
- 3) Through the depression of the activity coefficient of all the ions in the soil solution resulting in an increase in the solubility of normal crystalline compounds including phosphates that may be present in the soil.
- 4) Through a displacement of Ca ions by the cations of the salt leading to a precipitation of calcium phosphates at high pH.

We need therefore not be surprised if *A* finds the solubility of soil phosphates to be decreased by the addition of salts and *B* finds the solubility to be increased while *C* reports the salts to be without effect.

In relation to the several analytical methods, based on various phosphate solvents, which are now being tried, it is obvious that the principles here developed have an important

application. A study of the influence of citrates, lactates etc. on the amphoteric phosphates is now undertaken and will be reported in a later paper of this series.

Summary.

The paper deals with the effect of neutral salts on the amphoteric phosphates:

On the acid side of the I. E. P. (isoelectric point) there is a direct displacement of the phosphate ions by the anions of the salt. This leads to a cationic solvation and solution of the complex at a higher pH than in the absence of the salt. The SO_4 is more active than the Cl ion. The nature of the cation is here without effect.

At and above the I. E. P. of the complex and extending up to the I. E. P. of the free sesquioxide the salts cause a greater fixation of the phosphate ions. The fixation is dominated by the nature of the cations, the effect of K^+ being about twice that of Na^+ .

This fixation is ascribed to a depression of the hydrolysis and is explained as follows:

The slightly dissociated H ions of the phosphoric acidoid (acid residue) and OH ions of the sesquioxide basoid (basic residue) are displaced by the cations and anions of the salt according to the Donnan equilibrium. The saloids thus formed are highly or completely dissociated. This is in effect the same as if the acidoid and basoid had become stronger and must lead to a more extensive union between them or, what amounts to the same, to a depression of the hydrolysis.

The effect of the salt leads, therefore, to an increase in stability and a decrease in »solubility» of amphoteric precipitates of the type described. This is opposite to the effect of salts on normal precipitates (whose solubility is increased as a result of a depression in the activity coefficient of the ions in solution) and introduces a new concept in the theory of colloidal electrolytes which undoubtedly applies to all systems of *weak* acidoids and basoids.

Sammanfattning.

Arbetet handlar om neutralsalters effekt på de amfotära fosfaterna och fosfatfixeringen.

På den sura sidan om I. E. P. (isoelektriska punkten) förträngas fosfationerna direkt av saltets anioner. Detta leder till att en kationisk solvation och upplösning av komplexet inställer sig vid ett högre pH än vid frånvaro salt. SO_4 -ionen är härvid mera aktiv än Cl-ionen. Saltets kation har här ingen effekt.

Vid och ovanför komplexets I. E. P. upp till I. E. P. för den fria seskvioxiden åstadkomma salterna en ökad bindning av fosfationerna. Fastläggningen domineras av kationens natur; K-ionens effekt är omkring två gånger så stor som Na-ionens. Se fig. 45 och tab. 22.

Denna fixering tillskrives en nedsättning av hydrolysen och förklaras på följande sätt: De föga dissocierade H-ionerna hos fosforsyraacidoiden (syrest) och OH-ionerna hos seskvi-oxidbasoiden (basrest) förträngas av saltets kationer och anioner i enlighet med Donnan jämvikten. Den saloid, som då bildas, är starkt eller fullständigt dissocierad. Detta har samma effekt, som om acidoiden och basoiden hade blivit starkare och måste leda till en mera vidsträckt förening mellan dem, d. v. s. hydrolysen nedsättes.

Salteffekten ökar sålunda stabiliteten och minskar »lösligheten» för amfotära fällningar av den typ, som beskrivits. Detta är helt i motsats till salters effekt på normala fällningar, t. ex. kalciumfosfat. De normala fällningarnas löslighet ökar i närvaro av salter (jfr tab. 23), beroende på nedsättning av aktivitetskoefficienterna.

Det påvisade förhållandet att neutralsalter, när det gäller amfotära fällningar av den typ, som beskrivits, åstadkomma en ökad stabilitet och en minskad »löslighet» för dessa amfotära fällningar, introducerar en ny uppfattning i teorien för kolloidala elektrolyter, vilken otvivelaktigt gäller för alla system av *svaga* acidoider och basoider.

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The Influence of Temperature on the Permeability of Soils to Water.

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The forces which retard the movement of a liquid, e. g. water, are, as far as the movement is one-dimensional or laminar, only the forces of friction. Besides the friction against the walls of the vessel the friction within the liquid also plays a decisive part. This means that the viscosity of the liquid will strongly influence the movement.

If, on the other hand, the movement is of more than one dimension, i. e. turbulent, the forces of inertia will also appear as retarding forces. Thus, in this case, the forces of friction, and consequently, the viscosity of the liquid are not the only decisive factors of the movement.

From the foregoing it is evident that such factors as influence the viscosity of a liquid — among which temperature is a very important one — will have greater influence on the movement of a liquid when it is laminar than when it is turbulent. Thus, the movement of the liquid is more influenced by the temperature at the laminar than at the turbulent stage.

In the movement of ground water in the pores of soils there is no turbulence, the form of the movement is here, under natural conditions, laminar. The reasons of this are firstly that the velocity of the flow is here always low, secondly that

the pores of the soil are generally small, which two factors have the effect that Reynold's number does not exceed the value marking the boundary between laminar and turbulent movement. Because of this fact we may, therefore, expect a relatively strong dependence on temperature in the movement of ground water — stronger than in the movement of water in, let us say, a large tube.

This relation between temperature and the flow of ground water may be investigated from a purely theoretical point of view, as will be shown in the following pages. The object of this work is to compare the conclusions regarding the just mentioned relation that may be drawn on the basis of theoretical deduction with the data found by experiments. These data derive partly from the investigations of previous authors, partly from the writer's own experiments. Among those having made experimental researches into the problem may be mentioned HAGEN (1869), HAVREZ (1874), SEELHEIM (1880), HAZEN (1892), KRESNIK (1906), SCHAFFERNAK and DACHLER (1934). A further account of their works will be given in the following.

The theoretical influence of temperature on ground water movement.

The influence of viscosity on ground water movement. According to Newton (KAUFMANN 1931) the resistance of friction, m , occurring when two adjacent layers in a liquid move in relation to each other, is independent of a pressure perpendicular to the layers but directly proportional to their difference in velocity, dv , and inversely proportional to the distance between them, ds (see fig. 1). This may be expressed by the formula

$$m = \mu \cdot \frac{dv}{ds} \quad (1)$$

where μ is a constant of proportionality called the viscosity coefficient.

On the basis of equation (1) it is now possible to make the following deduction. In a tube with a circular section a flow of water is assumed to occur owing to an existing gradient of pressure. The form of movement of the water in the tube is assumed to be laminar, which is the case at sufficiently



Fig. 1.

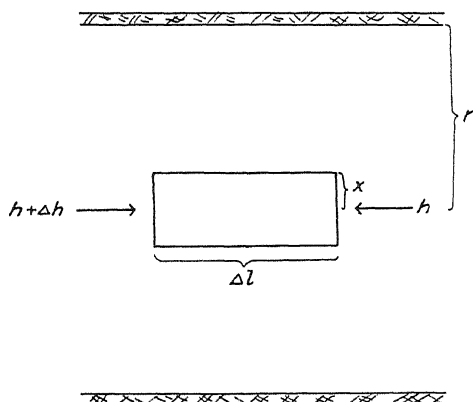


Fig. 2.

small flowing velocities and in tubes with small diameters. A cylindrical element of liquid with the radius x and the length Δl , situated so that its axis coincides with the length axis of the tube, is now considered. See Fig. 2. The difference in pressure between the end surfaces of the element, which is the cause of the movement of the element, is assumed to have the value Δh per superficial unit, i. e. for the end surfaces as a whole

$$\pi x^2 \cdot \Delta h.$$

This force is compensated by the force of friction, which for the whole mantle surface of the cylinder may be written by the aid of equation (1)

$$2 \pi x \cdot \Delta l \cdot \mu \frac{dv}{dx};$$

where v denotes the velocity of the flow.

This expression must be equal to the preceding one, and after fractioning we will have

$$x \cdot \frac{\Delta h}{\Delta l} = 2 \mu \cdot \frac{dv}{dx}. \quad (2)$$

Hence v may be obtained by integration

$$v = \int \frac{\frac{\Delta h}{\Delta l} \cdot x dx}{2 \mu}$$

$$v = \frac{\frac{\Delta h}{\Delta l} \cdot x^2}{4 \mu} + C$$

v is $= 0$ at the wall of the tube. If, therefore, we let r denote the radius of the tube, the above expression may be written

$$v = \frac{\frac{\Delta h}{\Delta l} (r^2 - x^2)}{4 \mu}. \quad (3)$$

The amount of water passing through a given ringsection of the tube is, for a continuous medium, equal to the product of the surface of the section and the existing velocity. If, therefore, the internal radius of the ringsection is x and the external radius $x + dx$, the amount of water, dQ , passing per unit of time, may be written

$$dQ = \frac{\frac{\Delta h}{\Delta l}}{4 \mu} (r^2 - x^2) 2 \pi x dx.$$

Hence the amount of water, Q , that flows through the whole tube, may be easily calculated

$$Q = \frac{\pi \cdot \frac{\Delta h}{\Delta l}}{2\mu} \int_{x=0}^{x=r} (r^2 - x^2) x dx = \frac{\pi \cdot \frac{\Delta h}{\Delta l}}{8\mu} r^4. \quad (4)$$

This equation, which has here been obtained theoretically, says that the quantity of water passing through a tube, when the movement is laminar, is proportional to the fourth power of the radius of the tube as well as to the prevailing gradient of pressure, and is further inversely proportional to the viscosity coefficient of water. The equation was constructed purely on empirical bases by HAGEN (1839) and by POISEUILLE (1843) and is therefore, by the suggestion of OSTWALD (1925), called the Hagen-Poiseuille law.

Equations of analogous meaning may be constructed for conduits with non-circular transverse sections.

As mentioned above, the movement of ground water is laminar, and therefore it might be expected at first sight that a law analogous to that of Hagen-Poiseuille would be applicable to this movement. The pores of the soil, however, are not straight as the imaginary conduit, and, moreover, their transverse sections vary from point to point. The way of a water particle through the soil mass will consequently not be linear, but will deviate continuously, which causes forces of inertia to enter. Nevertheless the law applying to the flow of water through the soil, which was established by DARCY (1856), shows agreement with the Hagen-Poiseuille law. It may be written

$$v = k \cdot \frac{dh}{dl} \quad (5)$$

where v is the velocity of the movement, k a constant and dh/dl the gradient of pressure. It seems that here just as in the Hagen-Poiseuille law, the velocity of the flow is proportional

to the existing gradient in pressure. If the forces of inertia appeared to any measurable extent, there would also be a quadratic term, which I have treated more closely in another work (GUSTAFSSON 1939). However, a great number of investigations have verified Darcy's law, from which follows that, compared to friction, the forces of inertia can play no important part.

Now, if the Hagen-Poiseuille law is applicable to the movement of free water through the soil, which thus involves that the existing forces of inertia are negligible compared to the forces of friction, it follows that the velocity of the flow, v , and consequently, also *the amount of water, Q , passing through the soil per unit of time, would be dependent on the viscosity of water in such a way that v , resp. Q would be directly proportional to the inverted value of the viscosity coefficient, μ .*

Hence it follows that the permeability of the soil would vary with temperature in the same way as the viscosity coefficient. In other words, the influence of temperature on the movement of ground water would be parallel to the influence of temperature on the viscosity of water.

• **The influence of the temperature on the viscosity.** That the viscosity of water varies with temperature was as early as 1798 observed by GERSTNER (1800). He proved by a series of tests the influence of temperature on the velocity of water flowing through capillary tubes.

There was, however, a rather long time before he had followers who made further investigations regarding the influence of temperature on the viscosity of water. They were, as mentioned above, HAGEN (1839) and POISEUILLE (1843), both of whom worked with varying temperatures when establishing their law. Poiseuille's results made it possible to formulate an equation for the influence of temperature on the velocity of water passing through capillary tubes, an equation which, according to the theory for this flow developed above

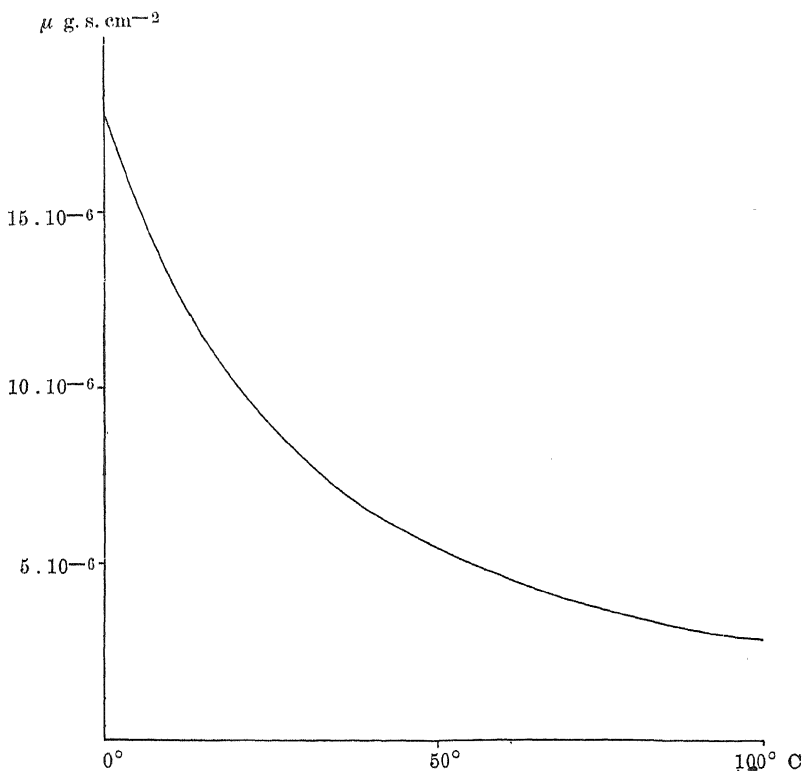


Fig. 3. The curve shows the variation of viscosity, μ , with temperature according to Poiseuille's equation.

coincides with the equation for the relation between temperature and viscosity coefficient. Poiseuille's equation is written

$$\mu = \frac{0.00001814}{1 + 0.0337 T + 0.00022 T^2} \quad (6)$$

where T is the temperature expressed in degrees C . In later investigations it has proved to give the relation between μ and T so well that no reasons of any great changes in it have occurred (HODGMAN 1933, p. 1064). The equation is illustrated by the curve in Fig. 3.

Now, if the reasoning presented above concerning ground water movement is true and the effect of the forces of inertia at the movement are negligible, the relation between temperature and the velocity of water in ground water movement must describe a similar curve.

The influence of temperature on ground water movement according to empirical data given by previous authors.

1. The oldest researches regarding the influence of temperature on the permeability of the soil were performed by HAGEN (1869). In his experiments he used a sand with a diameter of grain of about 0.3 mm. The experiments showed that the permeability at 23.5° R (29.4° C) and the permeability at 10° R (12.5° C) are in the ratio 3:2. This means that the increase in permeability for each degree C is about 3 %.

According to Poiseuille's equation (6) the viscosity coefficient has the value $12.46 \cdot 10^{-6}$ at $+12.5^{\circ}$ C and the value $8.32 \cdot 10^{-6}$ at $+29.4^{\circ}$ C. This means that $1/\mu_{29.4^{\circ}}$ is 49.76 % greater than $1/\mu_{12.5^{\circ}}$. The increase in temperature is $29.4^{\circ} - 12.5^{\circ} = 16.9^{\circ}$ C, which accordingly means that the change in the quantity $1/\mu$ is 2.94 % for each degree. It is evident that this change in $1/\mu$ corresponds very well to the change in the permeability of the soil found by HAGEN: 3 per cent for every increase in temperature by one degree. The relation is illustrated by the system of coordinates in Fig. 4, where Hagen's values form a dotted line, denoting the permeability at different temperatures, expressed in relative terms, the permeability at $+12.5^{\circ}$ being = 100. Further, the changes in the quantity $1/\mu$ have been registered in the same system of coordinates in the form of a full-drawn curve. $1/\mu$ is likewise expressed in relative terms with $1/\mu_{12.5^{\circ}} = 100$. We see that the two curves are practically coincident, which consequently means that the changes in permeability in the soil are parallel to the changes in the viscosity of water.

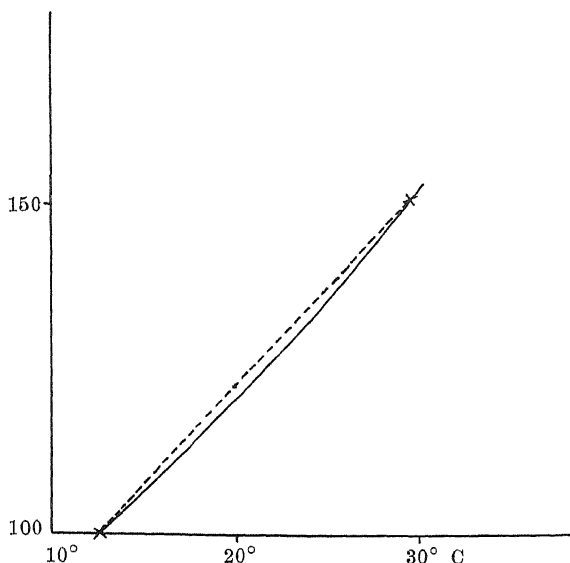


Fig. 4. The full-drawn curve shows the variation of the inverted values of viscosity with temperature. The dotted curve and the points represent the variation of permeability with temperature according to investigations by Hagen and Hazen. Only relative values are given.

The same result to which HAGEN arrived was later obtained by HAZEN (1892). He, too, found an increase in the permeability of the soil by 3 per cent for every rise in temperature of one degree according to the formula

$$w = 116 d_w^3 (0.7 + 0.03 T) \cdot I$$

where w is the velocity of the water, d_w the effective size of grain, T the temperature in degrees Celsius and I the existing gradient of pressure. Thus HAZEN's formula follows the dotted curve in Fig. 4.

2. The next investigation was carried out by HAVREZ (1874) who found in his experiments that the permeability of a sand is 6 times as great at a temperature of $+100^\circ \text{C}$ as at a temperature of $\pm 0^\circ \text{C}$. A calculation of the viscosity coefficient at these two temperatures according to Poiseuille's equation gives the results:

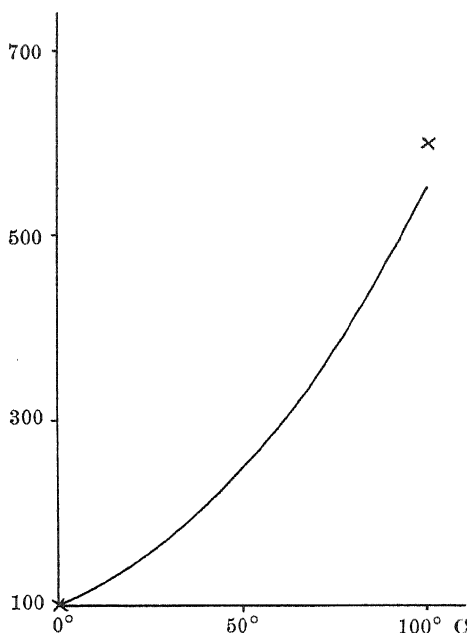


Fig. 5. The full-drawn curve shows the variation of the inverted value of viscosity with temperature. The dots show the permeability at 0° and 100° according to investigations by Havrez. Only relative values are given.

$$\mu_{100^{\circ}} = 3.26 \cdot 10^{-6} \text{ and } \mu_{0^{\circ}} = 18.14 \cdot 10^{-6}.$$

Hence it follows that

$$\frac{\mu_{0^{\circ}}}{\mu_{100^{\circ}}} = \frac{18.14}{3.26} = 5.57$$

i. e., the viscosity coefficient is 5.57 times as great at 0° C as at 100° C. We see that HAVREZ' experiments also indicate that the changes in permeability are parallel to the changes in viscosity. The results of HAVREZ' experiments have been expressed graphically in Fig. 5 in the same way as the data of previous workers.

3. The next investigation concerning the connection between temperature and permeability was performed in 1880

Table 1.

Temp.	μ	Permeability according to Seelheim cc/15 min.	$1/\mu$ rel.	Permeability rel.
9.0	$13.73 \cdot 10^{-6}$	94.0	98.3	100.0
10.0	13.35	96.0	101.2	102.1
11.0	13.00	98.0	103.9	104.3
12.0	12.63	100.0	106.8	106.4
13.0	12.29	102.5	109.9	109.0
14.0	11.97	105.5	112.9	112.2
14.5	11.81	106.5	114.1	113.3
15.0	11.66	108.0	115.8	114.9
15.5	11.51	109.5	117.4	116.5
16.0	11.37	111.0	118.8	118.1
17.0	11.18	114.0	120.9	121.3
18.0	10.81	117.0	124.9	124.4
19.0	10.55	120.0	128.0	127.6
19.5	10.42	122.0	129.7	129.7

by SEELHEIM (1880). Judging from the test reports, Seelheim's experiments seem to be made very carefully. For the determinations he used sand, which was first digested by concentrated hydrochloric acid to remove CaCO_3 and possibly soluble silicas, and then, after washing, boiled with soda. Then fusion was done with potassium bisulphate to remove the silt particles, further boiling with potassium hydroxide and, finally, igniting to remove organic matter. The sand, which had thus been exceedingly well rectified, was then put into a glass tube in which the determinations of permeability were performed. Unfortunately, Seelheim neglected to give the grain size of the sand.

The primary results of Seelheim's researches have been given in Table 1, in the third column from the left. In the second column the values of μ at the respective temperatures

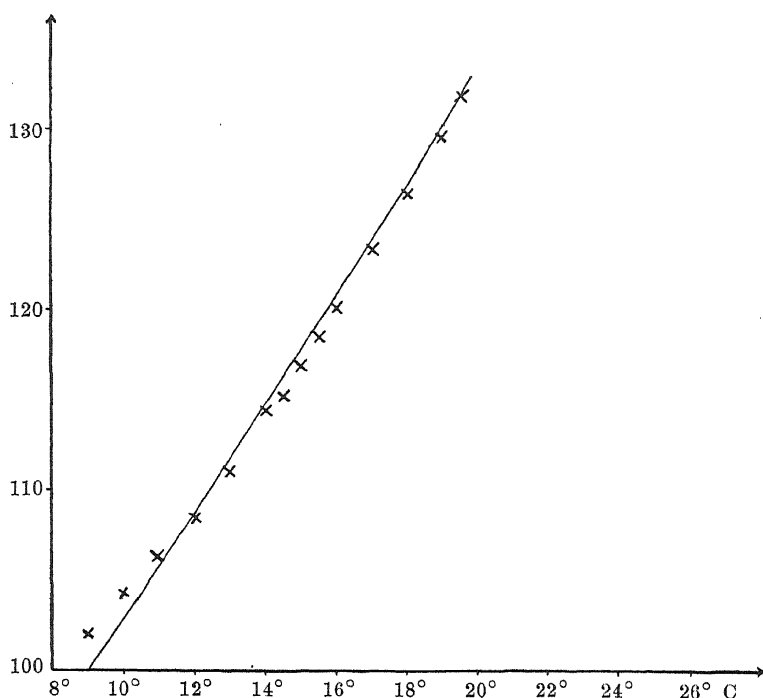


Fig. 6. The curve shows the variation of the inverted value of viscosity with temperature. The dots show permeability at different temperatures according to determinations by Seelheim. Only relative values are given. Cf. Table 1.

have been calculated. Finally, the two columns to the right give $1/\mu$ and the permeability, expressed in relative terms. These values are graphically presented in Fig. 6: $1/\mu$ by a full-drawn curve and the permeability by dots. The figure shows that the dots follow the curve almost perfectly. Accordingly SEELHEIM's tests show that the changes in permeability with temperature follow the changes in viscosity.

4. On the basis of a rather considerable experimental material, KRESNIK (1906) obtained the formula

$$f(t) = 1 + 0.047(t - 10) \sqrt[5]{d}$$

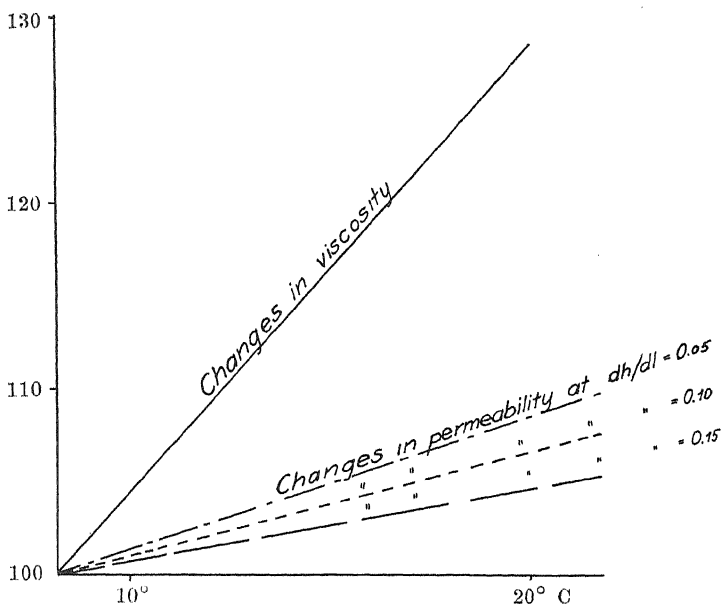


Fig. 7. The full-drawn curve shows the variation of the inverted value of viscosity with temperature. The dotted lines show the variation of permeability with temperature in a material with a grain size so great as to cause the movement of the water to become turbulent. According to Schaaffernak and Dachler. Only relative values are given.

for the influence of temperature, t , on permeability. d is here the grain size of the material, expressed in mm. On the basis of this formula he got another formula for the calculation of the »Normalfiltergeschwindigkeit», v_{10} , of a material, by which he understood the velocity of flow at 10° C. This formula is written

$$v_{10} = \frac{v}{f(t)} = \frac{v}{1 + 0.047(t - 10)\sqrt{d}}$$

where v is the velocity at a given temperature, t , and d as before, the grain size expressed in mm.

If this formula is applied to HAZEN's material, which, as mentioned above, had a grain size of about 0.3 mm, it is

found that, according to the formula, the velocity of flow would increase by 3.7 per cent for every rise of temperature of one degree. According to this, permeability would increase with temperature somewhat faster than the quantity $1/\mu$.

Moreover, according to KRESNIK's formula, the influence of temperature on permeability would increase with a greater grain size of the material. This has, however, not been verified by the investigations carried out by Schaffernak and Dachler, nor by the author's own experiments, as will be shown in the following.

5. SCHAFFERNAK and DACHLER (1934) determined the variation of permeability with temperature on two different materials, firstly a sand with a grain size of 0.18—0.20 cm, secondly gravel with a grain size of 0.6—0.7 cm. According to statements in the text of the treatise concerning the former material, the investigations indicated that permeability increases by about 3 per cent for every rise of temperature of one degree. Thus the result agrees with those of HAGEN and HAZEN, and the change in permeability follows the change in the viscosity of water according to Fig. 4. However, the author's statements in the text are not fully verified by the results of the measurements that have been published, which indicate that the changes in permeability would be somewhat more than 3 per cent for every degree C.

The results of the investigations on the coarser material are interesting. They indicate that temperature influences permeability considerably less here than in the former case. Furthermore, it appears that the influence of temperature varies with the prevailing difference in pressure, i. e. with the velocity of flow, in the following way within 10°—20° C:

Rel. difference in pressure	0.05	0.10	0.15
Increase in permeability in % at the rise of temperature of 1° C	1.00	0.68	0.52

A comparison between these values and the viscosity is made in Table 2.

Table 2.

Temp.	μ	$1/\mu$ rel.	Permeability expressed in rel. terms at the rel. diff. in pressure		
			0.05	0.10	0.15
10°	$13.35 \cdot 10^{-6}$	100.0	100.0	100.0	100.0
20°	$10.30 \cdot 10^{-6}$	129.6	110.0	106.8	105.2

The figures in the table are illustrated graphically in Fig. 7.

The reason why permeability is considerably less influenced by temperature in the tests with this coarser material is easily explained on the basis of the just presented theory. The cause is evidently that the flow becomes turbulent because the pores are so great that Reynold's critical number is exceeded when the velocity is low. With the occurrence of turbulence the internal friction of the liquid is no more the only resistance to the movement but also the forces of inertia play a certain rôle.

These forces make themselves felt the more, the greater the velocity, from which follows that the flow becomes in a corresponding degree less sensitive to the influences of temperature. The authors have also made experiments with this material with regard to the relation between difference in pressure and the velocity of flow. Also in these experiments the forces of inertia make themselves felt, the correlation being in this case not rectilinear.

The author's investigations.

Method. In the experiments arranged by the author in order to find out the variation of permeability with temperature, two different fractions of grain have been used: firstly coarse silt with a grain size of 0.2—0.6 mm, secondly gravel with a grain size of 4.0—6.0 mm. The fractions were chosen so that in the former, laminar movement might be expected

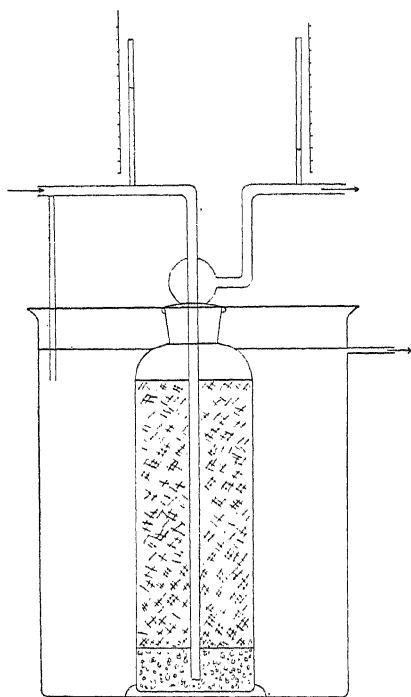


Fig. 8. Sketch of the apparatus used for the determination of the relation between permeability and temperature.

at the differences in pressure that were used, whereas, in the latter, turbulence was likely to occur. The fractions were prepared by sifting and were washed with water before the experiments begun.

For the tests a simple apparatus was used, built as shown by the sketch in Fig. 8. The fraction was placed in a bottle with a wide neck, closed by a tightly fitting glass-stopper. At the bottom of the bottle was a thin layer of gravel with a grain size considerably greater than that of the fraction intended for the investigation. The inlet tube extended down into the layer of gravel. The water entering through this tube was distributed through the gravel, and, having passed through the fraction, was led away by a tube connected with

the upper part of the bottle. At both the inlet and the outlet tubes there was a side tube for measuring the difference in pressure. The bottle was placed in a larger glass cylinder through which a permanent flow of water circulated. This water had the same temperature as that which passed through the sample. Owing to this arrangement the water suffered but a small change in temperature while passing through the soil.

Before the bottle was filled with material intended for the tests, the loss of pressure that was caused by the apparatus itself at different water velocities was investigated. It showed that these losses were very small compared to the loss of

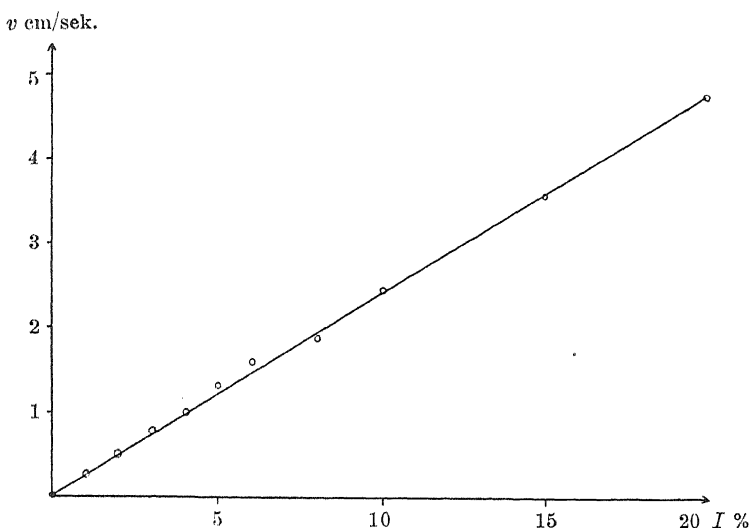


Fig. 9. Relation between the difference in pressure and the velocity of water in a material with a grain size of 0.2—0.4 mm. The relation is linear since the water movement is laminar.

pressure in the grain-fraction itself, because large tubes were used and, further, because the pores of the bottom layer were large. Consequently, the loss of pressure in the apparatus could be wholly neglected at the small velocities; at greater velocities, however, the values found had to be corrected with regard to this loss. This correction was made by help of a curve obtained by the calibration of the apparatus.

The relation between difference in pressure and the quantity of water passing per unit of time. The first measurement was made in order to find the relation between the existing difference in pressure and the quantity of water passing per unit of time. For the finer fraction this relation had been determined earlier by another apparatus. The result of this investigation, a closer report of which has been given in a previous work (GUSTAFSSON 1939) is expressed graphically in Fig. 9. It is here important to notice only that the existing

Table 3.

Pressure in mm column of water	Quantity of water cc pr min.	Pressure in mm column of water	Quantity of water cc pr min.
14	100	160	460
35	160	194	505
49	205	210	540
71	265	225	560
93	310	260	610
132	395	290	660

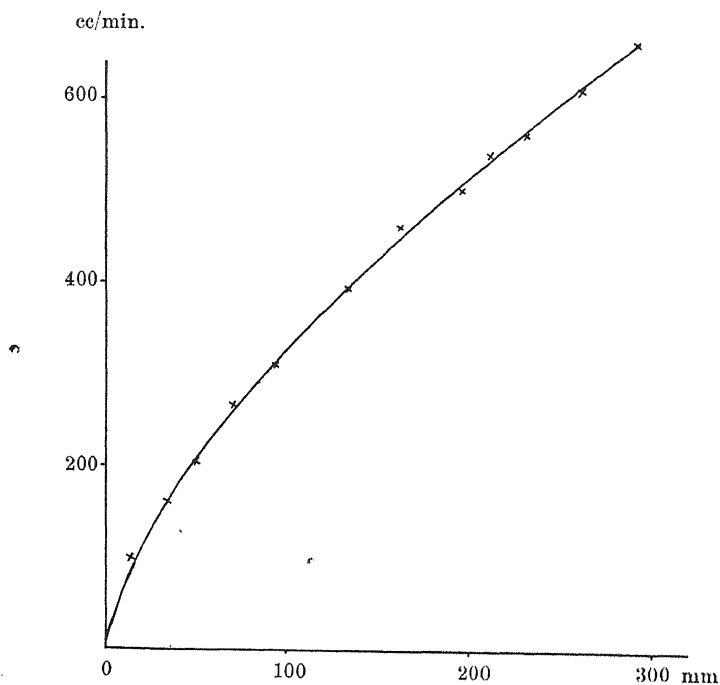


Fig. 10. Relation between difference in pressure and the quantity of water passing through a material with a grain size of 4—6 mm. Turbulence occurs, whence it follows that the relation is not linear here. Cf. Table 3. Temperature = 10° C.

Table 4.

Temp. C°	μ	cc/10 min.	$1/\mu$ rel.	Permeability rel.
9.0	$13.73 \cdot 10^{-6}$	68.0	100.0	100.0
10.5	13.16	69.5	104.3	102.1
11.0	13.00	72.5	105.6	106.9
12.0	12.63	72.0	108.7	106.0
13.0	12.29	77.5	111.7	113.9
13.5	12.13	78.0	113.2	114.5
14.5	11.81	78.5	116.3	115.4
15.5	11.51	80.5	119.3	118.6
16.5	11.23	85.0	122.3	125.0
17.5	10.95	84.5	125.4	124.3
18.0	10.81	89.0	127.0	130.8
18.5	10.68	90.0	128.6	132.1
20.0	10.30	91.0	133.3	134.0
21.0	10.05	94.0	136.6	138.1

relation is evidently linear, which agrees with DARCY's law and means that the current is laminar. On the basis of what has been indicated above, it may thus be expected that the variation of permeability with temperature will here be parallel to the change in viscosity.

The measurements of the relation between the difference in pressure and the quantity of water streaming through the coarser fraction per unit of time was made in the just described apparatus. The obtained results are reproduced in Table 3, the contents of which are given graphically in Fig. 10. Contrary to the result of the investigation with the finer material, the relation is here not linear, but is represented by a rather strongly bent curve. This indicates that DARCY's law does not apply to this case, because forces of inertia also appear owing to the turbulence. Here we might accordingly expect a smaller change in permeability with temperature than in the finer fraction.

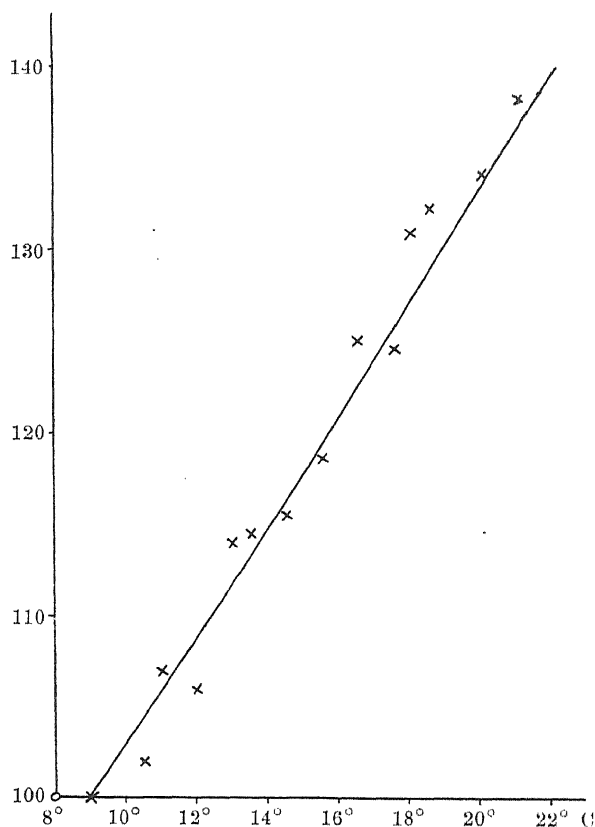


Fig. 11. The full-drawn curve shows the variation of the inverted value of viscosity with temperature according to Poiseuille's equation. The dots represent the permeability at different temperatures according to the author's investigations. Height of pressure 10 cm. Grain size of the material 0.2—0.4 mm. Only relative values are given. Cf. Table 4.

The relation between the prevailing temperature and the passing quantity of water. These tests were made in the apparatus just described. The determination was made firstly with the above mentioned silt, the grain size of which was 0.2—0.6 mm, secondly with the gravel, the grain size of which was 4—6 mm. The temperature was varied from 9°

Table 5.

Temp. C°	μ	cc/10 min.	$1/\mu$ rel.	Permeability rel.
9.0	$13.73 \cdot 10^{-6}$	137.0	100.0	100.0
10.0	13.35	129.0	102.8	94.2
11.0	13.00	144.0	105.6	105.1
12.0	12.63	146.0	108.7	106.6
13.0	12.29	154.0	111.7	112.4
13.5	12.13	159.0	113.2	116.1
14.0	11.97	157.0	114.7	114.6
14.5	11.81	159.5	116.3	116.4
15.5	11.51	159.5	119.3	116.4
16.0	11.37	174.0	120.8	127.6
17.0	11.18	181.5	122.8	132.5
18.0	10.81	180.0	127.0	131.4
20.0	10.30	180.0	133.3	131.4
21.0	10.05	194.5	136.6	142.0
22.0	9.82	200.5	139.8	146.4
24.0	9.31	203.5	147.5	148.5

to 24° C in the former case and from 9° to 40° C in the latter.

The experiments with the silt were carried out in 3 different series, and in each one of them the difference in pressure was kept constant and only the temperature was varied.

In the first series the difference in pressure within the column of earth was 10 cm. The obtained results are seen in Table 4. In the table is also inserted the viscosity coefficient of water, μ , at the different temperatures. In the two columns to the right the quantity $1/\mu$ as well as the permeability have been given in relative terms. In Fig. 11 the first mentioned values are reproduced in the shape of a full-drawn curve and the latter values in the shape of dotted curve. It is easily seen that permeability varies with temperature in the same way as viscosity.

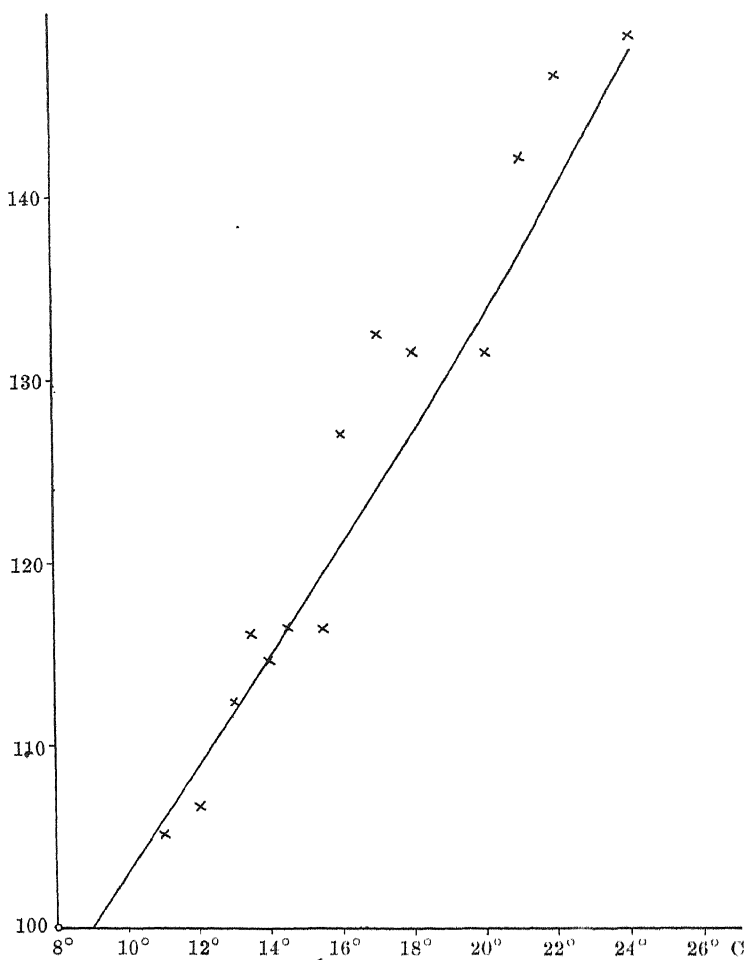


Fig. 12. The full-drawn curve shows the variation of the inverted value of viscosity with temperature according to Poiseuille's equation. The dots represent the permeability at different temperatures according to the author's investigations. Height of pressure 20 cm. Grain size of the material 0.2—0.4 mm. Only relative values are given. Cf. Table 5.

In the next series the difference in pressure within the column of earth was 20 cm. The results are given in Table 5 and have been illustrated graphically in the same way as the

Table 6.

Temp. C°	μ	cc/10 min.	$1/\mu$ rel.	Permeability rel.
9.0	$13.73 \cdot 10^{-6}$	201.0	100.0	100.0
10.0	13.35	210.0	102.8	104.5
11.0	13.00	208.5	105.6	103.7
12.0	12.63	212.5	108.7	105.7
13.0	12.29	215.0	111.7	107.0
14.0	11.97	222.0	114.7	110.4
15.0	11.66	231.0	117.8	114.9
16.0	11.37	240.0	120.8	119.4
17.0	11.18	256.0	122.8	127.4
18.0	10.81	270.0	127.0	134.3
19.0	10.55	268.0	130.1	133.3
20.0	10.30	274.0	133.3	136.3
21.0	10.05	280.5	136.6	139.6
22.0	9.82	276.5	139.8	137.6
24.0	9.31	301.0	147.5	149.8

previous series in Fig. 12. The third series had a difference in pressure of 30 cm, and the results of this series are given in Table 6 and Fig. 13. These two series give the same average result as the first, namely that temperature influences permeability in the same way as viscosity.

As we have seen, the results obtained from the material of 0.2—0.6 mm agree with the presented theory as well as with the results of earlier investigations.

The experiments with the gravel were made in two different series, in both of which the difference in pressure was kept constant.

In the first series the difference in pressure within the column of gravel was 5 cm. The results are seen in Table 7. To the table corresponds the graphic illustration in Fig. 14. The second series was performed with a difference in pressure of 15 cm. The results are to be found in Table 8 and Fig. 15.

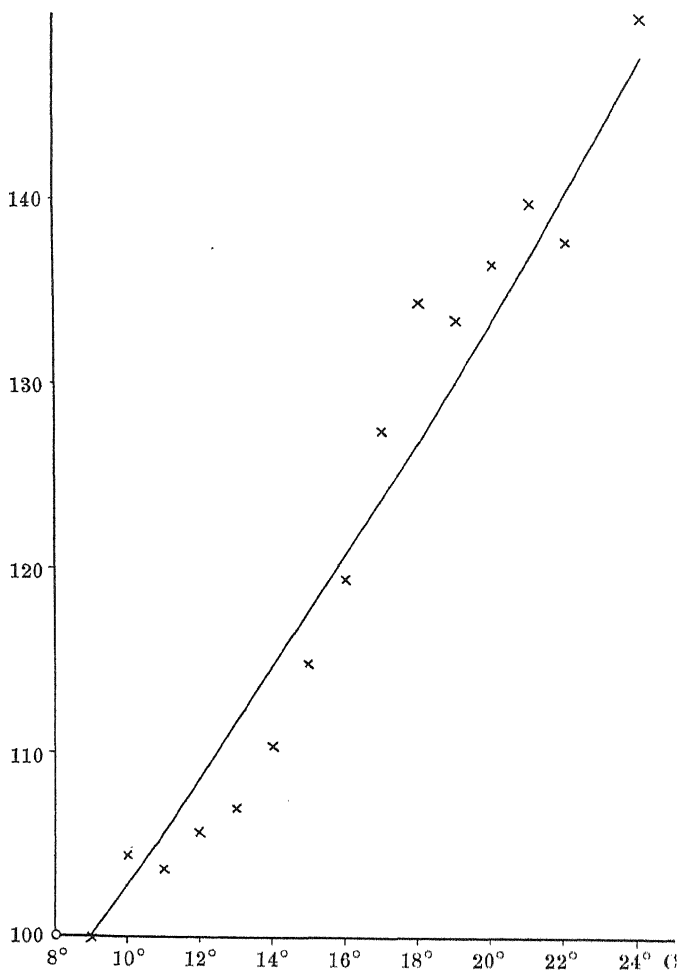


Fig. 13. The full-drawn curve shows the variation of the inverted value of viscosity with temperature according to Poiseuille's equation. The dots represent the permeability at different temperatures according to the author's investigations. Height of pressure 30 cm. Grain size of the material 0.2—0.4 mm. Only relative values are given. (Cf. Table 6.

From the graphic illustration in Figures 14 and 15 it is evident that the results from these two series differ from the preceding results. The influence of temperature on permeability is here considerably less than its influence on viscosity.

Table 7.

Temp. C ^o	μ	cc/10 min.	$1/\mu$ rel.	Permeability rel.
10.0	$13.35 \cdot 10^{-6}$	206	100.0	100.0
11.0	13.00	210	102.7	101.9
12.0	12.63	209	105.7	101.5
14.0	11.97	212	111.5	102.9
16.0	11.37	224	117.4	108.7
18.0	10.81	218	123.5	105.8
20.0	10.30	231	129.6	112.1
22.0	9.82	244	135.9	118.4
24.0	9.31	248	143.4	120.4
26.0	8.96	269	149.0	130.5
30.0	8.21	265	162.6	128.6
35.0	7.41	273	180.2	132.5
40.0	6.72	280	198.7	135.9

Table 8.

Temp. C ^o	μ	cc/10 min.	$1/\mu$ rel.	Permeability rel.
10	$13.35 \cdot 10^{-6}$	430	100.0	100.0
11	13.00	435	102.7	101.2
12	12.63	430	105.7	100.0
14	11.97	485	111.5	112.8
16	11.37	470	117.4	109.3
18	10.81	485	123.5	112.8
20	10.30	500	129.6	116.3
22	9.82	530	135.9	123.3
24	9.31	590	143.4	137.3
26	8.96	510	149.0	118.6
30	8.21	585	162.6	136.4
35	7.41	6.00	180.2	139.5
40	6.72	6.00	198.7	139.5

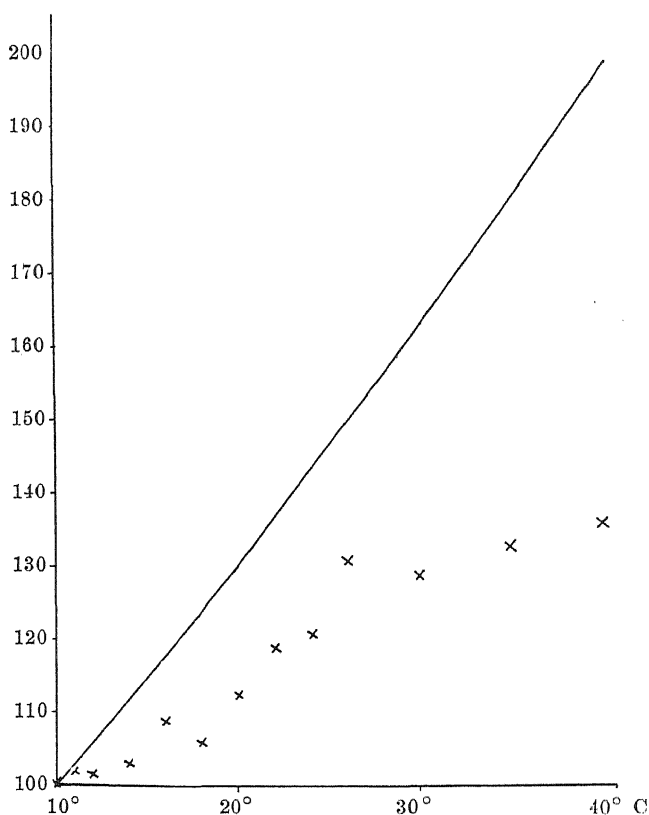


Fig. 14. The full-drawn curve shows the variation of the inverted value of viscosity with temperature according to Poiseuille's equation. The dots represent the permeability at different temperatures according to the author's investigations. Height of pressure 5 cm. Grain size of the material 4—6 mm. Only relative values are given. Cf. Table 7.

According to the adduced theory this is to be interpreted so, that in this coarse material there occur at the movement of the water not only *forces of friction* but also *forces of inertia*. This is due to the fact that turbulence enters into the movement owing to the coarseness of the pores. No changes in the influence of temperature on permeability with difference in pressure have, however, been observed.

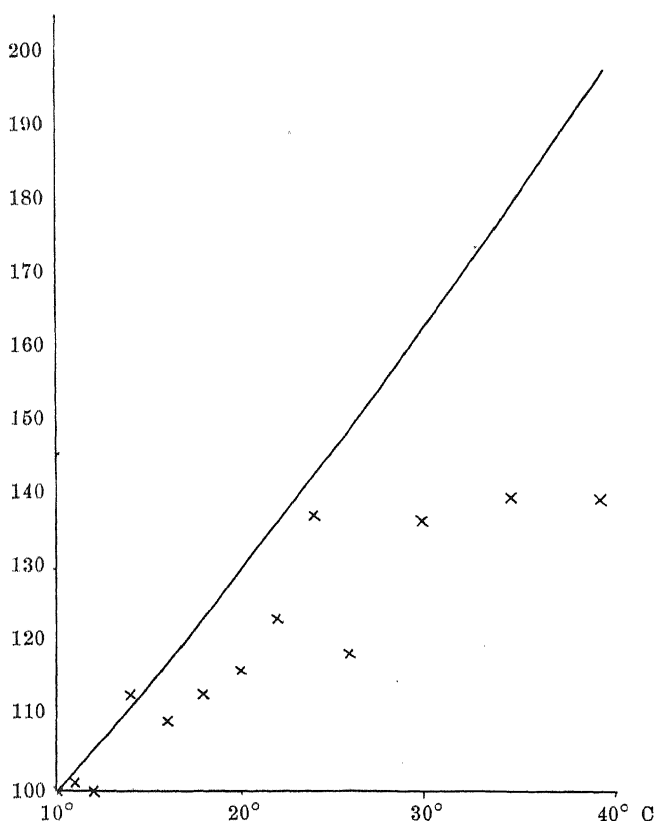


Fig. 15. The full-drawn curve shows the variation of the inverted value of viscosity with temperature according to Poiseuille's equation. The dots represent the permeability at different temperatures according to the author's investigations. Height of pressure 15 cm. Grain size of the material 4—6 mm. Only relative values are given. Cf. Table 8.

The importance of the influence of temperature for ground water movement under normal conditions.

As is well known, ground water from great depths below the ground surface is characterized by a relatively constant temperature. Measurements performed at the Institute of Agricultural Hydrotechnics of the Agricultural College of Swe-

den in connection with the use of ground water from the Uppsala ridge for hydraulic experiments have shown that the annual variation here does not exceed 2° C. Other measurements have given similar results (PRINZ 1919). It is therefore evident that the variation of temperature of this ground water is relatively insignificant. Any considerable variation in the state of flow owing to the influence of temperature will consequently not occur.

Nearer to the surface of the ground, however, conditions are different, which is worth noticing especially from the point of view of agriculture. The nearer to the surface, the greater are the changes in temperature. A periodic variation exists here, annual and daily. Near the ground surface we will therefore have a considerable variation of permeability on account of changes in temperature. Water due to the melting of snow in spring has a temperature slightly above 0° and must therefore move with much more difficulty than the water coming from a summer rain. If we assume a temperature of 1° C in the former case and 22° in the latter, the permeability on the two occasions may be expressed by the ratio 100:163. Thus there is a considerable difference in permeability.

From what has now been said follows further the necessity of taking the prevailing temperature into consideration at the determination of the permeability of a soil, whether this determination is made in the laboratory or by some field method. In earlier investigations this fact has generally been disregarded. To make the values obtained on different occasions commensurable it is necessary to reduce them to the same temperature. Since it has been shown here that the variation of permeability with temperature follows the variation of viscosity, it must be possible to make such a reduction on the basis of Poiseuille's equation (6). For this reduction of K from one temperature T_1 , to another T_2 we obtain the formula:

$$K_{T_1} = K_{T_2} \cdot \frac{1 + 0.0337 T_1 + 0.00022 T_1^2}{1 + 0.0337 T_2 + 0.00022 T_2^2}. \quad (7)$$

Not only the viscosity of free water varies with temperature, but also the viscosity of water bound by capillary and osmotic forces. Hence it follows that also the movement of such water is influenced by temperature. However, the relations are more complicated here than in the movement of free water, for here the driving forces which are of osmotic and capillary nature, are also dependent upon temperature. This is not the case in the movement of free water, where the driving power is gravitation. Thus the surface tension decreases with temperature, which means that the capillary force decreases. For the water that moves under the influence of capillarity, this implies that the decrease in viscosity with increasing temperature is compensated to a certain degree by a decrease in the force that brings about the movement, namely the surface tension. But the changes of surface tension with temperature are considerably less than the changes in viscosity, from which it follows that the velocity of capillary water increases with rising temperature, even though the increase is less than it is for free water. The capillary rise, however, is smaller at high temperatures than at low. What is now said is verified by experiments regarding the capillary rise performed in 1887 by Wollny (ZUNKER 1930, p. 115).

To what extent the osmotic movement of water is influenced by temperature can not be discussed more thoroughly, since there is no experimental material available.

In clay soils without crumb formations there is hardly any free water, since the greater part of it is governed by capillary and osmotic forces. Hence it follows that we can not expect the same dependence on temperature here as in the laminar movement of water in coarser soils. However, the experimental material available at present (e. g. that presented by SEELHEIM 1880) is not sufficient for a more thorough discussion of these questions.

Summary.

For the movement of water through a soil under natural conditions, Darcy's law holds true, which says that the velocity of the flow is directly proportional to the difference in pressure. This form of water movement is to be looked upon as laminar and the only force of resistance against the movement are the forces of inertia. The velocity of the flow will therefore be inversely proportional to the viscosity coefficient. Changes in the viscosity coefficient of the water, for instance on account of changes in temperature, will consequently cause the same percentage change in the permeability of the soil.

A closer examination of a series of results from older researches as well as of the author's own investigations clearly indicate that such is the case. Thus, according to Poiseuille's equation, every C° causes a change in the viscosity coefficient of about 3 per cent. The changes in permeability are on an average equally great.

In coarse soil fractions Reynold's number is exceeded at a certain velocity and a turbulent movement occurs. This means that besides the forces of friction the force of inertia also enters as a retarding force. Darcy's law is no more applicable, the relation between the difference in pressure and the velocity of the water being here represented by a curved line. Here, variations of the viscosity coefficient of water owing to changes in temperature can not be supposed to cause equal changes in permeability. The results of older work as well as the author's own investigations show that the changes in permeability are considerably less than the changes in viscosity.

The variation of temperature at greater depths in the ground is insignificant, and, therefore, there are no great changes in the velocity of ground water movement due to changes in temperature. Nearer to the surface, on the other hand, the permeability of the soil will be subject to considerable fluctuations on account of the variations of temperature.

The investigation shows how important it is that the results

of the determinations of the permeability of a soil — they may have been carried out in the field or by means of laboratory methods — are reduced to a fixed temperature in order to become commensurable. It is evident that such a reduction may be effectuated on the basis of Poiseuille's equation.

Sammanfattning.

Vid vattnets strömning genom jord gäller under naturliga förhållanden Darcy's sats, enligt vilken strömningshastigheten är direkt proportionell mot tryckfallet. Vattnets rörelseform är att uppfatta som laminär och såsom motståndskrafter mot rörelsen uppträda endast friktionskrafter. Strömningshastigheten blir därför beroende av vattnets viskositet, i det att den bör vara omvänt proportionell mot viskositetskoefficienten. Förändringar i viskositetskoefficienten hos vattnet exempelvis till följd av temperaturförändringar böra därför ge upphov till procentuellt lika stora förändringar i jordens permeabilitet.

En närmare granskning såväl av en rad äldre forskningsresultat som författarens egna undersökningar ge tydligt vid handen att så är fallet. Varje C° ger således enligt Poiseuilles ekvation upphov till en förändring i viskositetskoefficienten med 3 %. Förändringen i permeabilitet är i genomsnitt lika stor.

I grövre jordmaterial överskrides vid en viss hastighet det Reynoldska talet och turbulent rörelse inträder. Detta innebär att såsom retarderande krafter uppträda jämte friktionskrafterna även tröghetskraften. Darcy'ska satsen är ej längre giltig utan sambandet mellan tryckfall och vattenhastighet beskrives här av en kroklinje. Förändringar i viskositetskoefficienten hos vattnet till följd av temperaturförändringar kunna nu ej förväntas ge upphov till procentuellt lika stora förändringar i permeabiliteten. Äldre forskares resultat och författarens undersökningar visa också att permeabilitetsförändringarna äro avsevärt mindre än viskositetsförändringarna.

Temperaturvariationen är på större djup i marken obetydlig, varför några större förändringar i grundvattnets ström-

ningshastighet till följd av temperaturförändringar ej uppträda. Däremot torde jordens permeabilitet närmare ytan vara utsatt för avsevärda fluktuationer till följd av temperaturens växlingar.

Undersökningen visar vikten av att resultaten från bestämningar av jords permeabilitet vare sig de utförts i fält eller med laboratoriemetoder reduceras till en bestämd temperatur för att bli jämförbara. Undersökningen visar att en sådan reduktion kan ske med utgångspunkt från Poiseuilles ekvation.

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Scarification of hard-boiling peas.

By EWERT ÅBERG and G. BJÄLFVE.

(Preliminary Report.)

Communication No. 8 from the Institute of Plant Husbandry.

Among the tests in the practice course for the advanced students in plant husbandry, held in 1939 under the supervision of Professor HUGO OSVALD, boiling experiments on peas were carried out. The method employed was that which was worked out at the Ultuna branch station of the Swedish Seed Association. The majority of the pea samples derived from the institution's experimental field at Ultuna and from the Ultuna Farm. A number of samples were also kindly supplied by Docent R. TORSSELL.

In the case of certain of the samples, the boilings showed a very high proportion of hard-boiling peas. It could be assumed that a certain analogy with the hard seeds of red clover existed. Those samples containing hard-boiling peas were therefore used for scarifying experiments on the peas, before boiling. The peas were scarified in the dry state with a pointed knife, but only sufficiently to break through the integument. The peas were then boiled according to the principle mentioned above. The results are shown in table 1.

As will be seen from the table, the scarification had a very good effect. In the present case the percentage of partially boiled peas with 90 minutes' boiling-time decreased by at least 9 and at most 43. The material used in these experiments was, certainly, inconsiderable, as the tests were originally in-

Table 1.

Results of boiling tests on peas in the spring of 1939
(Resultat av kokningsundersökningar med ärter våren 1939.)

Boiling time 90 minutes; weight of samples 100 g.

(Kokningstid 90 minuter; provens vikt 100 g.)

Sample (Prov)	Treatment (Behandling)	Thousand- kernelweight (Tusenkörn- vikt) g	Number of peas per sample (Antal ärter pr prov)	Fully boiled peas (Färdigkokta ärter)		Partially boiled peas (Icke färdig- kokta ärter)	
				No.	%	No.	%
1	None (Ingen)	170	592	363	61	229	39
	Scarification (Rispning)		612	569	93	43	7
2	None (Ingen)	213	467	80	17	387	83
	Scarification (Rispning)		502	211	42	291	58
3	None (Ingen)	230	431	327	76	104	24
	Scarification (Rispning)		448	379	85	69	15
4	None (Ingen)	162	634	200	32	434	68
	Scarification (Rispning)		680	511	75	169	25

Sample 1: Torsdagsärt II, 1937 year's crop.

» 2: Torsdagsärt II, 1937 » »

» 3: Gyllenärt, 1938 » »

» 4: Ambrosiaärt II, 1937 » »

tended only as practice-tests for the course, but the results obtained seem worth reporting. They indicate that it should be possible to decrease the number of hard-boiling peas in a lot by scarifying or preparing the peas in apparatuses constructed on a principle similar to that of an ordinary seed-scarifier. The tests will be continued.

Sammanfattning.

Arbetets titel: *Risping av hårdkokta ärter.*

Vid den övningskurs för agr. lic. examen i växtodlingslära, som 1939 hölls under ledning av professor HUGO OSVALD, utfördes bl. a. kokningsundersökningar på ärter. Därvid användes den metod, som utarbetats vid Sveriges utsädesförenings baljväxtavdelning vid Ultuna. Flertalet ärtprover härstammade från institutionens försöksfält vid Ultuna och från Ultuna egendom. En del hade välvilligt ställts till förfogande av docent R. TORSELL.

Vid kokningarna visade det sig, att vissa prover innehöll en mycket stor mängd hårdkokta ärter. En viss analogi med hårdskalighet hos frön av rödklöver kunde förmodas föreligga. Dessa prov med hårdkokta ärter användes därför för försök med risping av ärtarna före kokningen. Ärtarna rispades i torrt tillstånd med en spetsig kniv och endast så mycket, att skalet blev genombrutet. Kokningarna utfördes därefter enligt samma princip som ovan, och resultaten framgå av ovanstående tabell.

Som synes av tabellen, har rispingen haft mycket god verkan. I föreliggande fall har procenttalet icke färdigkokta ärter pr 90 min. kokningstid nedgått med lägst 9 och högst 43. Det är visserligen ett obetydligt material, som ingått i dessa undersökningar, vilka från början endast voro avsedda som övningsuppgift under kursen, men de resultat, som erhållits, synas vara värda att delgivas en vidare krets. De antyda, att man skulle kunna minska procenthalten hårdkokta ärter i ett parti genom att rispa eller preparera ärtarna i apparater, konstruerade i likhet med en vanlig fröpreparator. Undersökningarna komma att fortsättas.

Viviparous forms of *Agrostis*.

By EWERT ÅBERG.

Communication No. 9 from the Institute of Plant Husbandry.

There seem to be no viviparous forms of *Agrostis* described from Scandinavia. Such forms are mentioned by HEGI (1935) in his »Illustrierte Flora von Mitteleuropa». He states that viviparous forms are found within *Agrostis stolonifera* L. and *Agrostis tenuis* Sibth. A short account of two viviparous forms found in Scandinavia will be given here.

During a journey to Northern Scandinavia in the summer of 1935 the author collected a number of *Agrostis* plants, which were then grown at Ultuna in the experimental field of the Institute of Plant Husbandry. Among these were two viviparous plants. One of them (Plant I) was collected at Abisko and belonged to *Agrostis tenuis* and the other (Plant II) was collected at Narvik and belonged to *Agrostis stolonifera*.

Plant I was found on dry, sandy soil near the railway station at Abisko. It was characterized by erect growth and moderately broad leaves. The lowest ligules were strongly anthocyanin coloured and the flags were erect when the panicles emerged. The plant did not flourish at Ultuna and died during the winter 1937 to 1938. Consequently it was only studied during the summers 1936 and 1937. The panicles that appeared were few, but they were all distinctly viviparous (Fig. 1).

Plant II was collected at the road-side near the railway station at Narvik. It was very well developed. The leaves

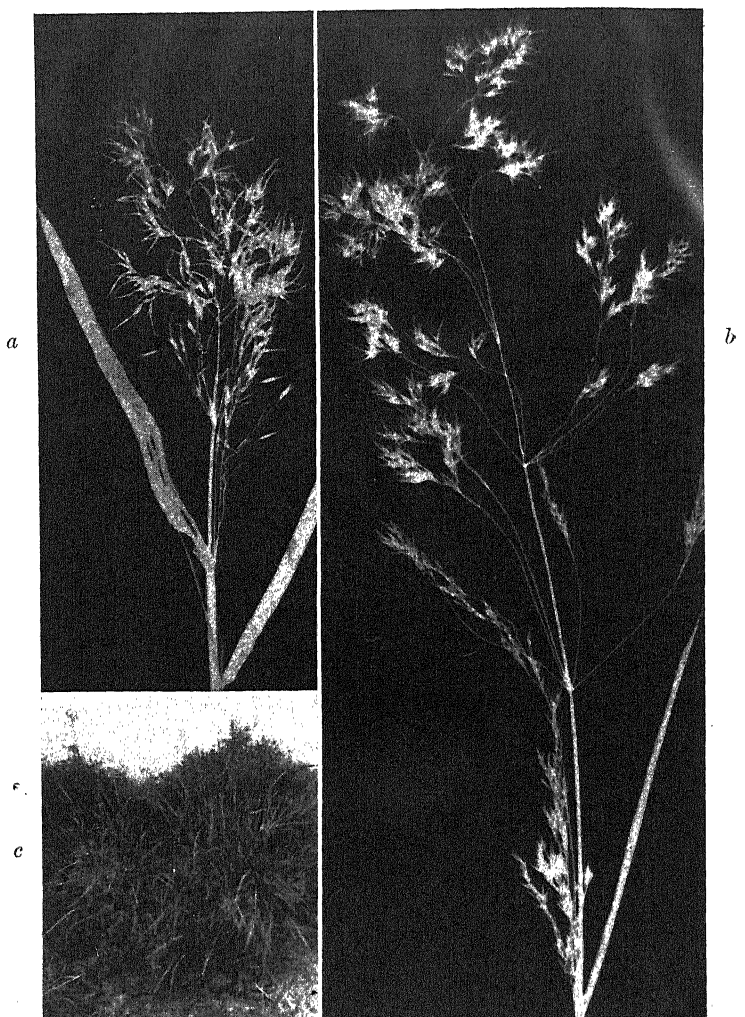


Fig. 1. Panicles of plant I (a) and plant II (b) $\times \frac{3}{4}$. Mode of growth of plant II (c).

were dark green, and the mode of growth was procumbent. When planted at Ultuna the tuft was divided into two parts. These thrived very well and were studied during the summers

1936 to 1939. During this period the following characters for the two plants have been noted. Mode of growth procumbent, tillering very good, spread through subterranean runners considerable, leaves dark green, flags erect when the panicles emerge. A great number of panicles appeared, and they were viviparous. The plant is still flourishing in the experimental field and will be subject to further investigations. Fig. 1 shows the mode of growth and a panicle of the plant.

Sammanfattning.

Arbetets titel: *Vivipara former av Agrostis*.

Vivipara former av *Agrostis* synas ej tidigare ha beskrivits från Skandinavien. Författaren påträffade under en resa till Norra Skandinavien 1935 två plantor av *Agrostis*, som vid odling på Ultuna visade sig vara vivipara. Den ena insamlades i Abisko och tillhörde *Agrostis tenuis* Sibth. den andra insamlades i Narvik och var en *Agrostis stolonifera* L.

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